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Specialization of a calmodulin-like protein: androcam adopts a single conformation over the entire physiological range of calcium

by

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ABSTRACT

Specialization of a calmodulin-like protein: androcam adopts a single conformation over the entire physiological range of calcium

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The ubiquitous and highly conserved calcium binding protein calmodulin exhibits structural plasticity, broad target binding specificity and the ability to tune its affinity for Ca^{+2} ion. Collectively, these properties enable calmodulin to transduce biological calcium signals to hundreds of downstream targets. Despite the versatility of calmodulin, metazoans express many essential calmodulin-like proteins that perform tissue specific functions. In this thesis, I have studied androcam, an essential protein in *D.melanogaster* that is 67% identical to calmodulin, to determine how its structure, Ca^{+2} binding and target recognition properties differ from those of calmodulin and contribute to its unique function. My NMR structures solved at high and low calcium show that unlike calmodulin, which switches its conformation in response to changes in $[Ca^{+2}]$, each lobe of androcam is locked in a single fold over the entire physiological range of $[Ca^{+2}]$.

The androcam C-lobe has two EF hands which each ligand a Ca^{+2} ion, and is structurally similar to calmodulin. However, it binds Ca^{+2} much tighter than calmodulin and is therefore constitutively present in the Ca^{+2} bound "open" conformation that potentiates interactions with hydrophobic targets. The N lobe of androcam does not bind Ca^{+2} at physiological concentrations but is well structured and adopts a "closed" conformation similar to Ca^{+2}-free calmodulin that is not expected to bind a hydrophobic anchor. Consistent with these structural observations, chemical shift

perturbation experiments show that androcam interacts with the unique 'Insert2' peptide of the biological target Myosin VI with its C lobe only, whereas calmodulin binds this target using both lobes. Our results indicate that the androcam sequence has been optimized by evolution starting from the highly versatile calmodulin sequence to adopt only one of the many conformations that calmodulin can sample. We propose that many other calmodulin-like proteins might have also evolved to be specialists for a unique functional state out of the plethora of conformations that calmodulin has been shown to populate.

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Terms, Symbols, and Abbreviations

2D	two dimensional
3D	Three dimensional
$^3J_{HNHA}$	H^N-H^α through bond coupling
$^3J_{CC\alpha}$	aliphatic-α ^{13}C-^{13}C through bond coupling
$^3J_{CC}$	aliphatic-carbonyl ^{13}C-^{13}C through bond coupling
$^3J_{CN}$	^{13}C-^{15}N through bond coupling
Å	angstrom, 10^{-10}m
ARIA	Ambiguous Restraints for Iterative Assignments
apo	free of ligand, e.g. calcium ion
C	backbone carbonyl carbon
C_α	α carbon
Ca^{+2}	Calcium ion
CBCA(CO)NNH	3D NMR experiment that correlates H^N,N,C_α and C_β resonances
COSY	Correlation spectroscopy
CSI	Chemical shift index
DTT	Dithiothreitol
EDTA	ethylenediaminetetraacetic acid
H_α	α proton
HN	Amide proton
HNCA	3D NMR experiment that correlates H^N,N and C_α resonances
HNCO	3D NMR experiment that correlates H^N,N and C resonances
holo	bound to ligand, e.g. calcium
HSQC	Heteronuclear single quantum coherence
Hz	Hertz, frequence units in cycles sec^{-1}
IPTG	Isopropyl β D-1 thiogalactopyranoside
N	amide nitrogen
NMR	Nuclear magnetic resonance
NOE	Nuclear overhauser effect
NOESY	Nuclear overhauser enhancement spectroscopy
OD_{600}	Optical density at 600 nm
PAGE	Polyacrylamide gel electrophoresis
PMSF	Phenylmethylsulfonylflouride
ppm	parts per million
rms	root mean square
SDS	Sodium dodecyl sulfate
TSP	3-(trimethylsilyl)-propionic acid-d4, sodium

1. INTRODUCTION

1.1 Calcium signaling

The paucity of cytosolic Ca^{+2} allows it to be used as a messenger molecule. Cytosolic calcium is maintained at very low concentrations (1 – 100 nM) in a resting cell and can approach 1 μM in an excited cell. The extracellular calcium concentration is however very high at 2 mM (Bootman and Berridge, 1995). Since Ca^{+2} cannot be metabolized like other secondary messengers, cells have developed various types of regulatory, buffer and extrusion proteins to maintain low cytosolic [Ca^{+2}]. Sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps sequester excess cytosolic Ca^{+2} into the endoplasmic reticulum (ER) lumen where it is stored in bound form to buffer proteins such as calsequestrin (Clapham, 1995). Ca^{+2} signaling has evolved to find application in a variety of cellular activities like muscle contraction, cell cycle regulation, and cytoskeleton assembly. Activities such as muscle contraction and gene transcription can be initiated by a simple localized increase in [Ca^{+2}], whereas activities such as neurotransmitter release, gap junction regulation, cell migration, and lymphocyte activation are caused by more complex patterns of Ca^{+2} waves and oscillation (Tsien and Tsien, 1990). Ca^{+2} enters the cell either through voltage dependent / ligand gated non selective Ca^{+2} channels or is released from intracellular stores such as the ER (through IP3 receptors), sarcoplasmic reticulum (through ryanodine receptors) or mitochondria (Nowycky and Thomas, 2002).

The cause of many diseases can be directly attributed to Ca^{+2} signaling gone awry. Genetic defects in the calcium sensor calpain cause muscular dystrophy and type 2 diabetes (Carafoli, 2002). Malfunctioning of ryanodine receptors (RyR) of the

sarcoplasmic reticulum causes rigid muscles leading to malignant hyperthermia (Carafoli, 2002). Early onset of Ca^{+2} signaling in acinar cells releases trypsin storage granules prematurely that causes acute pancreatitis (Parekh, 2000). As per the calcium hypothesis of Alzeheimer's disease (Khachaturian, 1989), the disruption of Ca^{+2} signals primarily triggers apoptosis leading to neuronal degeneration. The central role of Ca^{+2} signaling in biological systems and its importance in health and disease have made understanding this signal transduction system a major question in modern biological and medical research.

1.2 Calcium binding proteins

Many cytosolic proteins have evolved specialized Ca^{+2} binding regions to respond to influxes of calcium, most common is the EF hand motif (Clapham, 1995). Calcium binding proteins can be classified as regulatory and buffering proteins (Ikura, 1996). Regulatory proteins bind Ca^{+2} with a prominent conformational change whereas buffering proteins bind Ca^{+2} tighter than their regulatory counterparts but with little conformational change. The conformational change that occurs in regulatory proteins upon Ca^{+2} binding furnishes them with modulatory and signaling functions. Many regulatory and buffering proteins contain EF hands and differences in these EF hands render them capable of a range of different conformational changes upon Ca^{+2} binding, leading to the observed variety of biological responses including the activation of specific set(s) of targets.

Calcium signaling proteins take part in a variety of biological functions. Calcium released from intracellular stores activates calmodulin dependent protein kinase IV, a multifunctional serine-threonine kinase that activates the nuclear transcription factor

cAMP responsive element binding protein (CREB) that is responsible for T-cell activation (Sheng et al., 1991) (Anderson and Kane, 1998). The process of fertilization induces intracellular Ca^{+2} waves and oscillation patterns that trigger specific developmental signaling pathways (Stricker, 1999). In the brain, calcium signaling activates the kinase CaMKII, a structural modification in which forms the basis of memory storage (Carafoli, 2002). Nuclear Ca^{+2} signaling involves the activation of a variety of kinases (e.g MAP kinase) and phosphatases that regulate the cell cycle (Santella and Carafoli, 1997). In contrast to the preceding biological processes that include long time scale responses, a rapid depolarization over the sarcolemma of cardiac muscle cells is required for the huge burst of Ca^{+2} leading to a heart beat (Clapham, 1995). Ca^{+2} efflux from the SR through RyR and IP_3R receptors cause K^+ and Cl^- channel activity influencing smooth muscle contraction (Wary, 2005). Intercellular Ca^{+2} signaling also modulates ciliary movement in epithelial cells for efficient mucus removal, wound healing, information processing in the brain, regulating blood flow in vascular endothelial cells, and contact inhibition through gap junctions to prevent the trigger of cancerous growth (Sanderson et al., 1994).

1.3 Calmodulin

Calmodulin (CaM) is one of the chief mediators of intracellular Ca^{+2} signaling responsible for transducing Ca^{+2} concentrations into a desired biological outcome. Calmodulin is involved in a myriad of cellular activities such as signaling, development, cytoskeleton assembly, kinase-phosphatase reactions and ion channel function. It has been highly conserved during evolution; the *Drosophila* calmodulin is only three amino

acids different from vertebrate calmodulin. Calmodulin is a soluble 148 residue acidic protein with distinct N and C terminal lobes separated by a long and flexible central linker. Each lobe contains two Ca^{+2} binding EF hand motifs separated by a short anti-parallel β sheet (Babu et al., 1985). The binding of Ca^{+2} to acidic side chains and a backbone carbonyl in both lobes of calmodulin exposes a target binding hydrophobic cleft. The central linker of calmodulin can adopt a helical conformation but also unwinds to give an elongated structure. The conformational changes of the lobes together with the flexibility of the central linker allow calmodulin to interact with a very diverse range of protein targets with nM or even pM affinity in a wide variety of binding architectures. Some binding modes exhibited by calmodulin are shown in Figure 1.2 and brief descriptions are listed in Table 1.1.

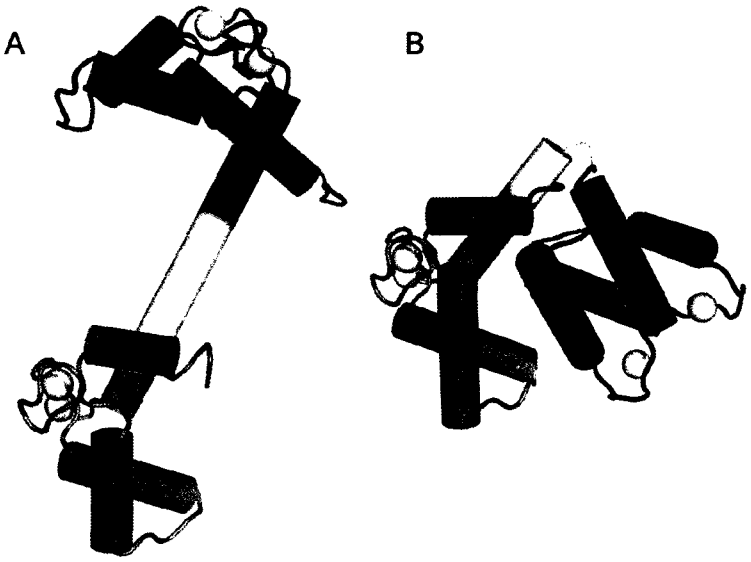


Figure 1.1 Structures of Ca^{+2}-calmodulin in extended and collapsed forms

Calmodulin has distinct N (cyan) and C (blue) terminal lobes that each binds two calcium ions (yellow spheres) and are connected by a central linker. A. Crystal structure of *Drosophila* Calmodulin in extended form with the linker as a rigid α helix (4CLN: Taylor et al, 1991). B. Crystal structure of bovine calmodulin in collapsed form with the linker as a flexible loop. (1PRW: Fallon et al, 2003). Despite the large differences in the architecture of the whole protein, each lobe closely superimposes on itself in both structures. RMSD of N-lobe Cα superpositions (helix-helix from residue 6-64) = 2.01 Å. RMSD of C-lobe Cα superpositions (helix-helix from residue 83-140) = 0.78 Å .

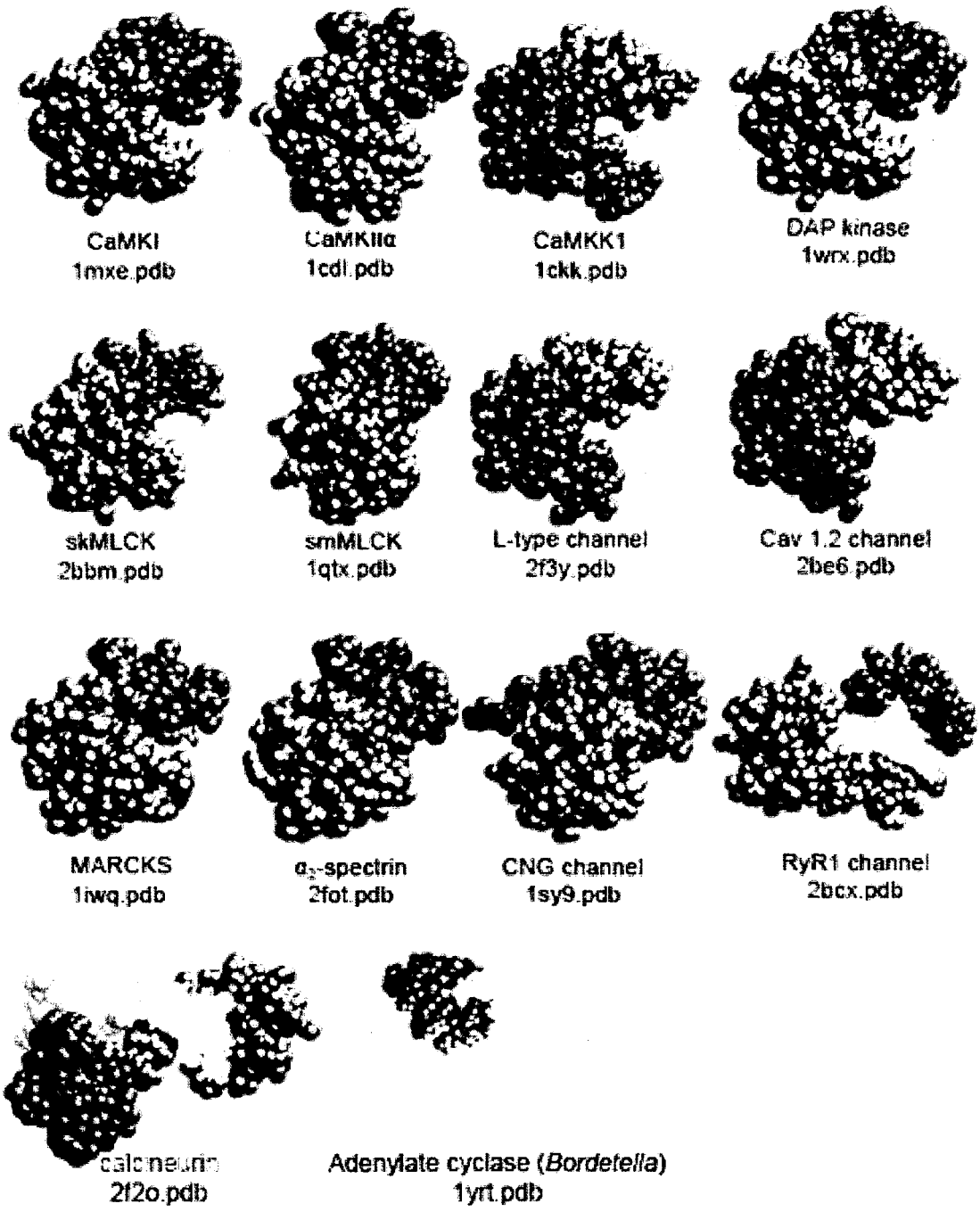


Figure 1.2 A selection of binding modes exhibited by calmodulin

Calmodulin bound to various peptide or protein targets shown as space filling models. Calmodulin residues 1-75 are shown in blue and 76-148 are shown in red. Target peptides or proteins are shown in yellow. From Boscheck et al, Biochemistry (2008) 47, 1640-1651.

Table 1.1 Common target binding modes exhibited by calmodulin

Calmodulin target	Description of binding mode
Basic Amphiphilic α helical (BAA) peptides	The most commonly observed binding mode of CaM, e.g. skeletal muscle myosin light chain kinase (MLCK) (Ikura et al., 1991) and smooth muscle MLCK (Roth et al., 1991) and CaM KIIα (Meador et al., 1993) . A 1:1 calmodulin-peptide complex that adopts a helical conformation with anti-parallel orientation (N-C & C-N), i.e. N terminal of peptide binds C lobe CaM and vice versa. This binding complex is alternatively named after the spacing of the hydrophobic anchor residues on the peptide as a 1-5-10 or a 1-8-14 binding mode.
CaMKK	(Osawa et al., 1999): The peptide contacts CaM in parallel orientation (N-N & C-C) and forms a helix followed by a β sheet between the hydrophobic pockets of the two lobes. The binding mode is 1-16.
CaMBD of K+ channel	(Schumacher et al., 2001): The CaM binding domain forms a dimer with two CaM molecules each of which is bound to three helices. N lobe of CaM is bound to Ca^{+2}, C lobe is Ca^{+2} free.
ATP activated plasma membrane Ca+2 pump	(Elshorst et al., 1999): only the C lobe of CaM binds the peptide (C20W), the peptide adopts a shorter α helix and makes three hydrophobic contacts with the C lobe
Anthrax toxin adenylyl cyclase of of *Bacillus anthracis* and *Bordetella pertusis*	(Drum et al., 2002): The Ca^{+2} bound C lobe and Ca^{+2} free N lobe of CaM bind in a completely novel mode with extended conformation. Both lobes are able to interact independent of each other
Voltage gated calcium channels	(Van Petegem et al., 2005) Ca^{+2} channels $Ca_v1.2$ have the consensus IQ sequence (IQxxxRGxxxR) for CaM binding. The all helical peptide forms a 1:1 complex with parallel orientation (C-C & N-N). Ca^{+2} bound C lobe of CaM is rigid but the Ca^{+2} bound N lobe adopts two different conformations.

Additional interaction modes may be quite distinct or variants on a theme. Structural studies in the MacKenzie lab on calmodulin complexed with a target peptide from the ryanodine receptor (RyR) of sarcoplasmic reticulum have shown that the two Ca^{+2}-CaM lobes bind the helical RyR peptide hydrophobic anchors at a novel 1-17 spacing (Maximciuc et al., 2006); this is analogous, but structurally distinct from, the standard Basic Amphiphilic Alpha-helical (BAA) binding modes (Rhoads et al, 1997). NMR data for Ca^{+2}-free calmodulin in complex with the same peptide show that only the

C lobe anchors while the N lobe is free in solution, emphasizing the plasticity of calmodulin recognition modes.

Calmodulin in its Ca^{+2}-free (apo) form is also able to interact with targets such as plasma membrane calmodulin trap neuromodulin (Strittmatter et al., 1990), phosphorylase b kinases (Cohen et al., 1978) and the actin binding brush border myosin I (Cheney and Mooseker, 1992). The IQ motif of unconventional myosins is the most well known consensus sequence for apo-CaM binding (Cheney and Mooseker, 1992). The motif is also present in many important biological molecules such as cytoskeletal proteins (Connexins, TrnI, Synaptotagmin), cell cycle regulators (GTPases) and the Protein kinase C substrate Neuromodulin (Rhoads and Friedberg, 1997). The IQ motif found at the carboxy terminal of voltage dependent Ca^{+2} channels imparts a lobe specific function to calmodulin (Van Petegem et al., 2005).

As undoubtedly the most important EF hand protein, calmodulin is able to act as a messenger in many of the Ca^{+2} signaling pathways that mediate diverse biological functions. Calmodulin activates kinases such as Myosin Light Chain Kinase (MLCK) by binding to the target autoinhibitory region in response to Ca^{+2} and causing a conformational change that exposes the kinase active site (Hu et al., 1994).

1.4 Calmodulin-like proteins in biology

Calmodulin is ubiquitously expressed and highly conserved, and it exhibits a high degree of structural flexibility and recognizes a wide variety of targets. Despite this multifaceted and versatile nature of calmodulin, other Ca^{+2} binding EF hand proteins that

are quite similar to calmodulin have been discovered that perform specialized functions in certain cell types. To enumerate a few:

Troponin C: is the Ca^{+2} sensor responsible for skeletal and cardiac muscle contraction. Like calmodulin, it has distinct N and C lobes separated by a helical liker. The C lobe EF hands bind Ca^{+2} tighter (100nM) than the N lobe (10μM) and also exhibit affinity for Mg^{+2}. In relaxed muscles, the C lobe is bound to Mg^{+2} and it is the low affinity N lobe that plays a role in Ca^{+2} signaling during excitation. (Gordon et al, 2000).

Human Calmodulin Like Proteins (hCLPs) are found in epithelial cells of exclusively breast, cervix, prostate and skin and are down regulated in tumor cells of these tissues. (Rogers et al., 2001).

CML24 is 40% identical to calmodulin and contains four functional EF hands and a somewhat longer flexible linker, is expressed in most *Arabidopsis thaliana* tissues, and influences the decision to flower and the response of the plant to pathogens (Delk et al., 2005). Fifty other calmodulin-like proteins exist in *Arabidopsis thaliana* (McCormack et al., 2005)

EhCaBP, a Ca^{+2} binding protein from the protozoan parasite *Entamoeba hystolica*, contains four functional EF hands, has a short but flexible 8 residue central linker, and exposes a larger hydrophobic pocket in the C lobe upon Ca^{+2} binding than calmodulin (Atreya et al., 2001).

Guanylate Cyclase-Activating Protein (GCAPs) are calmodulin-like proteins with four EF hands, although the first EF hand does not bind Ca^{+2}; GCAPs regulate the activity of

retinal guanylate cyclases which are responsible for sensitivity over a broad range of light intensities (Ames et al., 1999).

Recoverin is a retinal rod outer segment protein with four EF hands, but the first and fourth do not bind Ca^{+2}; recoverin inhibits rhodopsin kinase (Flaherty et al., 1993).

Calerythrin is involved in Ca^{+2} signaling in *Streptomyces erythreus*; it contains four EF hands, but the second does not bind Ca^{+2} (Tossavainen et al., 2003).

It is known that mutations in the highly conserved calmodulin sequence can affect Ca^{+2} binding affinity, induce conformational changes and alter target specificity (Maune et al., 1992). Many of the calmodulin-like proteins identified to date have sequence changes in the EF hand regions (relative to calmodulin) that render them less competent or incapable of Ca^{+2} binding. One possible result of evolutionary changes in EF hand residues of calmodulin-like proteins would be an altered sensitivity to calcium; these calmodulin variants could serve specialized biological functions or provide responses that differ from those of calmodulin. Exactly how changes in amino acid sequence might induce structural changes in calmodulin-like proteins and how these variants would bind targets in order to impart such specific regulatory functions, perhaps under special cellular conditions, is not clear. In a broad sense, the aim of my thesis work is to understand how sequence variation in such paralogs gives rise to structures and binding properties distinct from those of calmodulin. In this work, I have studied the testis-specific calmodulin-like protein "**Androcam**", which is essential to the process of spermatogenesis in *D. melanogaster*. Our results indicate that the androcam sequence has been optimized by evolution starting from the highly versatile calmodulin sequence to

adopt only one of the many conformations that calmodulin can sample. We propose that many other calmodulin-like proteins might have also evolved to be specialists for a unique functional state out of the plethora of conformations that calmodulin has been known to populate.

1.5 Androcam

Androcam is a *Drosophila melanogaster* calmodulin-like protein that was discovered in Dr. Kate Beckhingam's laboratory at Rice University. The protein is detected exclusively in testis by immunofluorescence microscopy, and the transcripts are found in adult male testis but not in female flies (Frank et al., 2006). Androcam is well conserved between *D. melanogaster* and *D. pseudoosbcura*, which are separated by 50 million years of evolution, suggesting that a conserved biological function is supported by the androcam protein sequence, which is 67% identical to calmodulin (Pavlik et al., 2006). Androcam acts as a myosin VI light chain and is essential to the process of spermatogenesis (Frank et al., 2006) (see Figure 1.3).

The motor protein myosin VI plays an essential role in stabilizing the movement of actin cones in the process of individualization during spermatogenesis. Myosin VI has two twenty residue sequence motifs, called 'Insert 2' and 'IQ domain', which are known to bind calmodulin in mammalian cells (Menetrey et al., 2005). In *Drosophila* ovaries, calmodulin binds to myosin VI and co-immunoprecipitates with it, but despite the testis expressing three times as much calmodulin than androcam, it is androcam and not calmodulin that co-immunoprecipitates with Myosin VI (Frank et al., 2006). Immunomicroscopic studies have also shown that it is androcam and not calmodulin that selectively co-localizes with myosin VI at the front of actin cones in developing sperm tails (Frank et al., 2006) (Figure 1.4). An androcam RNAi experiment done in Dr. Beckingham's laboratory has established that lack of sufficient androcam results in improperly developed actin cones and sterile males (Figure 1.5, courtesy of Dr. Beckingham).

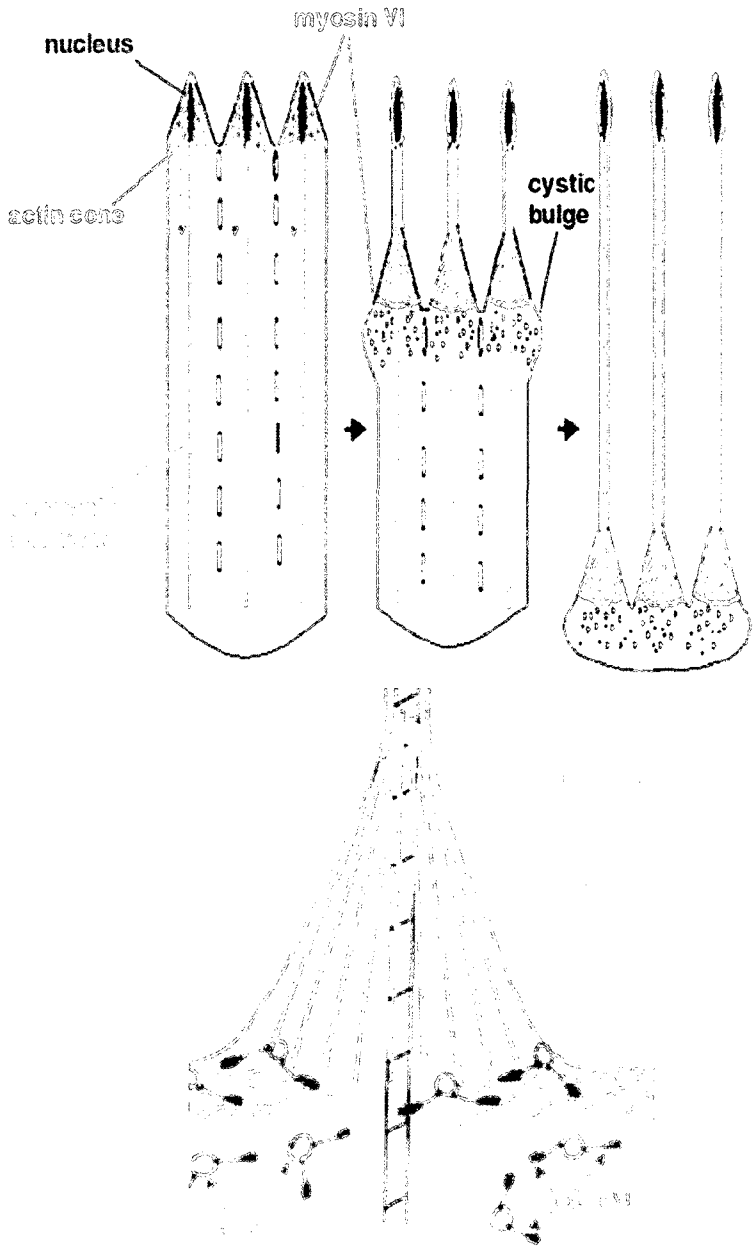


Figure 1.3 Spermatogenesis in *Drosophila melanogaster*

The individualization of sperm in *D. melanogaster* involves the wrapping of plasma membrane around the axoneme by stripping out cytoplasm and other cell organelles. The wrapping is accomplished by the movement of an actin cone stabilized by the motor protein myosin VI. Calmodulin is a known light chain for myosin VI in mammalian cells. However, in the testis of *D. melanogaster*, it is androcam and not calmodulin that co-localizes and co-precipitates with myosin VI. Model diagram from Hicks et al., 1999 and Noguchi et al., 2003

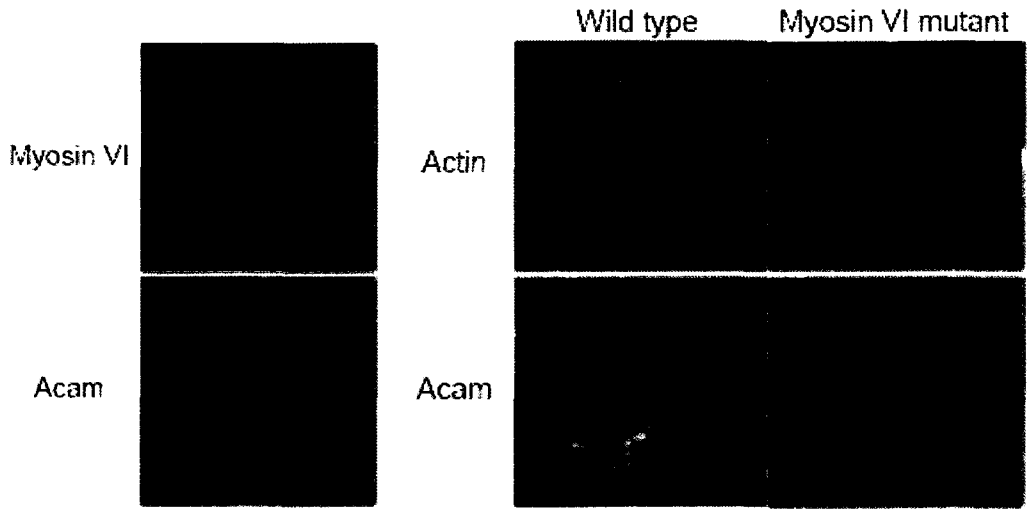


Figure 1.4 Androcam co-localizes with myosin VI at the front end of developing actin cones.

Mutants with highly reduced expression levels for myosin VI, show very low ACaM co-localization with actin. Left panel: immunostaining of myosin VI (red) and androcam (green) at the leading edge of developing actin cones in wild type spermatocytes. Right panel : transverse(lateral) view of wild type and myosin VI mutant spermatocytes immunostained with actin (red) and androcam (green). Myosin VI expression is needed for localization of androcam at the front end of actin cones and proper cone formation. From Frank et al., 2006.

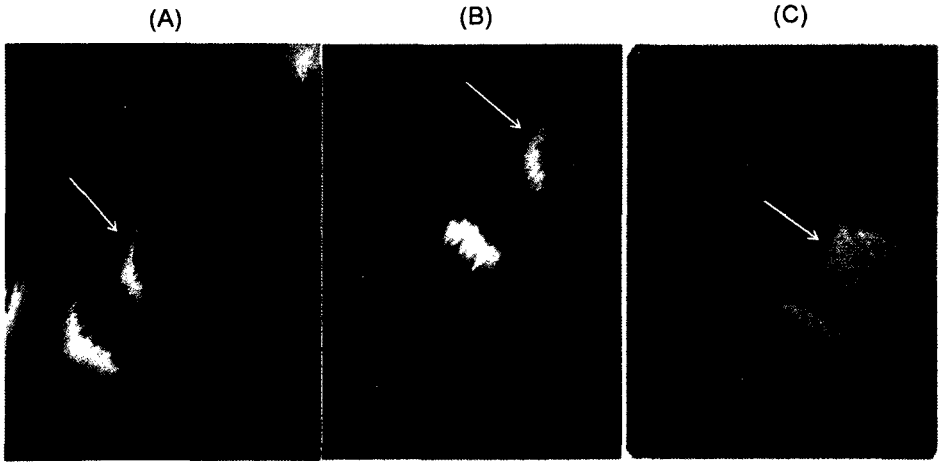


Figure 1.5 RNAi knockdown of androcam gives sterile flies with incomplete individualization and double set of actin cone formation.

Reducing the expression levels for androcam results in improper cone formation. Panels A, B, and C correspond to early, middle, and late stages of spermatogenesis. The white arrow indicates the leading set of actin cones. The actin cones in the absence of androcam are poorly developed and do not span the entire width of the cystic bulge. Some repair in cone formation happens over time and a trailing set of properly developed cones seem to appear but nevertheless fail to accomplish individualization and result in sterile male flies. Figure is courtesy of Dr. Beckingham, unpublished data.

One of the four putative androcam Ca^{+2} binding EF hands is defunct (Martin et al., 1999), as would be expected based on differences in the primary sequences of the calmodulin and androcam EF hands (see Figures 1.6 and Figure 1.7).

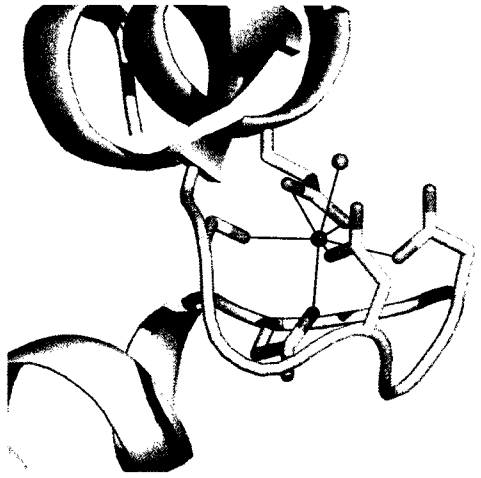


Figure 1.6 Ca^{+2} coordination in a canonical EF hand motif

The loop region of the fourth EF hand in calmodulin (PDB 1CLL (Chattopadhyaya et al., 1992). Ca^{+2} is coordinated as a pentagonal bi-pyramid. D129, D131, and D133 contribute a single side chain O atom. Q135 coordinates Ca^{+2} with its backbone carbonyl oxygen and E140 is a bi-dentate ligand contributing both side chain O atoms. The seventh coordination is completed by a water molecule. Oxygen atoms are shown in red and the Ca^{+2} ion in green.

```
          1                                                   37
ACaM MSELTEEQIAEFKDAFVQFDKEGTGKIATRELGTLMR
CaM  ADQLTEEQIAEFKEAFSLFDKDGDGTITTKELGTVMR
         38                                                   74
ACaM  TLGQNPTEAELQDLIAEAENNNNGQLNFTEFCGIMAK
CaM   SLGQNPTEAELQDMINEVDADGNGTIDFPEFLTMMAR
         75                                                  ---
ACaM  QMRETDTEEEMREAFKIFDRDGDGFISPAELRFVMIN
CaM   KMKDTDSEEEIREAFRVFDKDGNGFISAAELRHVMTN
         112                                                 148
ACaM   LGEKVTDEEIDEMIREADFDGDGMINYEEFVWMISQK
CaM    LGEKLTDEEVDEMIREADIDGDGQVNYEEFVTMMTSK
```

Color code for Ca^{+2} liganding residues ● Side chain O⁻ ● Backbone C=O ● Bi-dentate glutamate

Figure 1.7 Sequence comparison of calmodulin and androcam

Sequence alignment of androcam and calmodulin. Residues of the Ca^{+2} liganding EF hand regions are aligned and shown in color. Residues that potentially ligand Ca^{+2} with a single side chain O are in green, the ones contributing a backbone C=O are in red and the bi-dentate glutamate is in blue.

The biophysical properties of androcam and its binding affinities for calcium (Table 1.2) were found to be very different from calmodulin (Martin et al., 1999): the C-lobe of androcam binds Ca+2 more tightly than calmodulin, but the N-lobe binds more weakly than calmodulin (see Table 1.2).

Table 1.2 Calcium affinities of calmodulin and androcam (Martin et al., 1999)

	Ca^{+2} Binding constants (K_D)			
	C lobe EF hands		N lobe EF hands	
	K_1	K_2	K_3	K_4
CaM	0.38 μM	5.88 μM	4.6 μM	47 μM
ACaM	25 nM	56 nM	>80 μM	-

Competitive titration of 25 μM calmodulin or androcam was done in presence 28.5 μM 5,5' Br_2 BAPTA. The Ca^{+2} binding constants were determined as described by Linse et al, 1991. The binding of the third site in androcam is close to the measurable limit and thus is 80 μM or weaker.

The affinities of androcam and calmodulin for 'IQ' and 'insert 2' wild type and mutant target peptides were also found to differ (Martin et al., 1999). Together, these results indicate that androcam is not only functionally but structurally and biochemically distinct from calmodulin. The biochemical and structural differences between androcam and calmodulin can be related to the need for a special kind of myosin VI light chain (provided by androcam and not calmodulin) during the individualization of spermatids in the *Drosophila* testis. My thesis work asks: How different is androcam from calmodulin structurally? How might these differences influence androcam Ca^{+2} (and target) binding properties? How does the versatility of calmodulin structure and function serve as a basis for the adaptation and evolution of calmodulin-like proteins that serve more narrowly defined biological roles?

2. PREPARATION OF ANDROCAM NMR SAMPLES

2.1 Expression construct and glycerol stock optimization

I obtained the pET15b androcam (ACaM) expression vector used for previous biophysical studies (Martin et al., 1999) from Dr. Beckingham's laboratory. This vector contains a cloning artifact in which the native second residue, serine, is replaced with alanine. I mutated this residue back to serine using PCR mutagenesis and confirmed the entire ACaM coding sequence by automated dideoxy sequencing. I then transformed the plasmid into BL21(DE3) expression cells and made seven glycerol stocks at OD_{600} = 0.4. To check for protein expression, I grew all seven glycerol stocks in 5 ml of Terrific Broth until they reached OD_{600}=1.0 and then induced with 5 μL of 0.5 M IPTG. Five hours after induction I harvested the cells and resuspended them in 100 mM KCl, 1 mM EDTA, 50 mM TrisHCl pH = 7.5, 1 mM DTT. I froze the cell resuspensions at -20°C, followed it by thawing to room temperature and treated them with lysozyme (1:1000), sonicated and digested them with DNase1 (1:10000). 15% Tris-HCl precast polyacrylamide gels and Coomassie Blue staining were used to check protein expression. Glycerol stock 6 showed the highest protein expression and was used in all further androcam protein sample preparations.

2.2 Androcam leaches out of *E. coli* cells upon freeze-thawing

Although I initially prepared ACaM according to an established protocol (Martin et al., 1999), I noticed that when BL21(DE3) cells expressing androcam are frozen and thawed in lysis buffer, ~95% of the androcam simply leaches out of the cells into the

resuspension solution whereas most high molecular weight proteins remain in the pellet. Thus upon centrifuging the thawed cell resuspension, I could begin with a supernatant that had most of the expressed androcam in very high purity (Figure 2.1). I thus decided to avoid the lysozyme treatment, sonication and DNase I digestion steps that are part of the standard preparation of ACaM (or calmodulin) to prevent contamination with endogenous *E. coli* proteins. Neither multiple freeze-thaw cycles nor expediting the thawing process improves the yield of extracted ACaM. I treated 100 ml of supernatant with 20 µl of 100 mM phenylmethylsulfonyl fluoride (PMSF) to inhibit proteases. Androcam expressed in minimal media leaches upon freeze-thawing to a similar extent as when expressed in rich media.

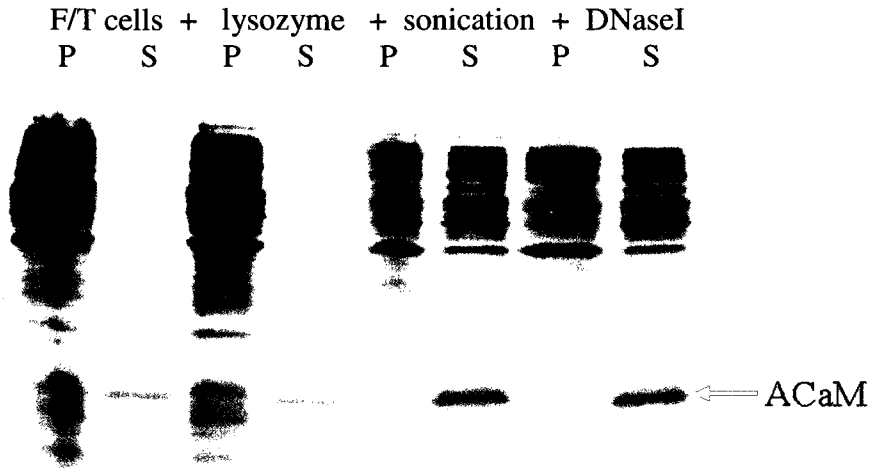


Figure 2.1 Androcam leaches out of *E. coli*

15% Tris-HCl acrylamide gel showing the extent of androcam leaching after steps of freeze-thaw, lysozyme treatment, sonication, and DNase I digestion for a terrific broth expression. 200 µl aliquots taken at each step were centrifuged, supernatant separated and pellet resuspended in equal amount of water. P: pellet resuspension. S: supernatant. 9 µl of sample is loaded into each well.

2.3 Concentration and fractionation using $(NH_4)_2SO_4$

I treated the Freeze/Thaw supernatant to $(NH_4)_2SO_4$ precipitation steps to separate ACaM from contaminating endogenous *E. coli* proteins on the basis of the tendency to aggregate in the presence of a chaotrope due to hydrophobic interactions. A saturated $(NH_4)_2SO_4$ stock solution of 70g/100ml (weight/volume ratio) was prepared and lower strengths were obtained by diluting with cell resuspension supernatant. A survey from 30% to 80% saturation in the absence of Ca^{+2} revealed that 40% saturation precipitates some of the contaminating proteins but leaves androcam in solution (Figure 2.2, first lane). Ca^{+2} binding to androcam is expected to cause conformational changes that expose hydrophobic pockets, making it more prone to aggregation. As seen in Figure 2.2, lane 2, addition of Ca^{+2} causes some androcam to precipitate out at 40% saturation; at 65% saturation nearly all of the androcam precipitates out in the presence of endogenous proteins. When working with the supernatant of a 40% $(NH_4)_2SO_4$ cut performed in the absence of Ca^{+2}, highly purified androcam is obtained by addition of Ca^{+2} and making the solution to 80% saturation as can be seen in the right panel of Figure 2.2.

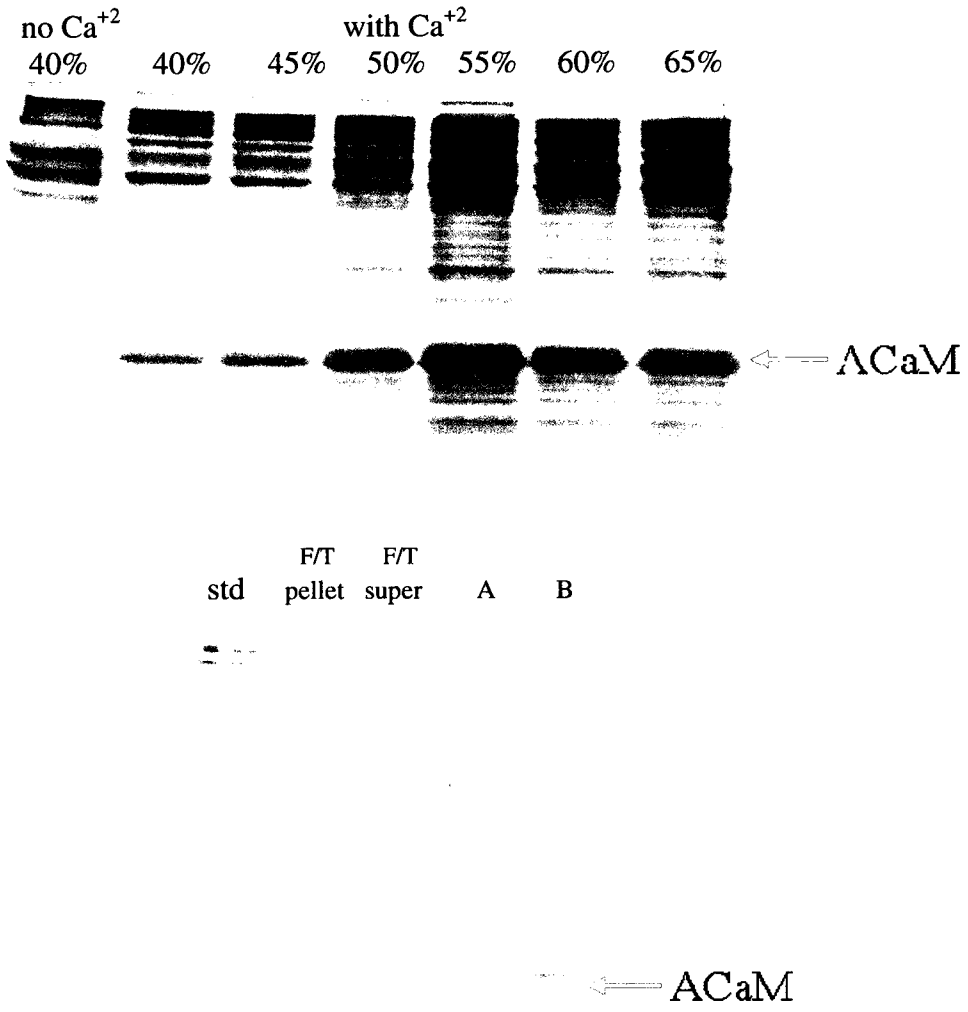


Figure 2.2 Ammonium sulfate precipitation of freeze-thaw supernatant of androcam

Upper Panel: 15% Tris-HCl PAGE showing the yield of androcam starting from a supernatant obtained by freezing and subsequently thawing the cell resuspension (F/T) and subjecting it to increasing ammonium sulfate concentration in the presence of calcium. Each lane is loaded with the pellet obtained after saturating with the indicated amount of $(NH_4)_2SO_4$.
Lower Panel: Lane A) pellet obtained after saturating the F/T supernatant of androcam made upto 40% in $(NH_4)_2SO_4$ in the absence of calcium. Lane B.) pellet obtained after the supernatant of 40% $(NH_4)_2SO_4$ is made upto 80% in $(NH_4)_2SO_4$ with addition of calcium.

2.4 Purification using hydrophobic interaction columns (HIC)

After the ammonium sulfate cut, androcam is further purified on hydrophobic phenyl sepharose columns (5 ml HiTrap™ Phenyl FF, GE Healthcare, Cat:17-5193-01) using the AKTAprime Fast Protein Liquid Chromatography (FPLC) system from (Amersham Pharmacia Biotech, Cat:18-1135-24). The following buffers are used on the hydrophobic interaction columns:

HIC-B1: 1 M KCl, 2 mM EDTA, 25 mM Tris, 10 mM DTT, pH 7.4

HIC-B2: 1 M KCl, 10 mM $CaCl_2$, 25 mM Tris, 10 mM DTT, pH 7.4

HIC-B3: 500 mM KCl, 10 mM $CaCl_2$, 25 mM Tris, 10 mM DTT, pH 7.4

HIC-B4: 500 mM KCl, 2 mM EDTA, 25 mM Tris, 10 mM DTT, pH 7.4

This method uses two HIC steps that exploit the Ca^{+2}-dependence of androcam hydrophobicity. I equilibrated Phenyl column 1 with HIC-B1, resuspended the androcam-containing pellet $(NH_4)_2SO_4$ pellet in HIC-B1, and loaded it at 1 ml/min onto the column. In high salt (1 M KCl), many endogenous proteins bind to phenyl sepharose, but androcam is in the flow-through because in the presence of EDTA no hydrophobic pockets would be exposed. I then added $CaCl_2$ to make the androcam-containing flow-through fractions 10mM in Ca^{+2}, rendering androcam hydrophobic. I loaded this mixture at 1 ml/min onto Phenyl column 2 that was pre-equilibrated with HIC-B2; in this step androcam binds. I followed the initial load with three column volumes of HIC-B2 and then washed the column with ten volumes of HIC-B3, which drops the salt concentration by 50% (though still in the presence of calcium) and elutes contaminating proteins. In the final step, androcam is eluted in the presence of EDTA by washing column 2 with HIC-B4. The elution profiles and SDS-PAGE of selected fractions from the phenyl

columns are shown in Figure 2.3. The HIC-B4 fractions that predominantly contain androcam are pooled together and dialyzed to 25 mM KCl, 2 mM EDTA, 25 mM TrisHCl, 10 mM DTT for further purification on an ion exchange column.

2.5 Purification using ion exchange (Q) chromatography

The quaternary amine anion exchange column HiTrap™ Q XL, (5x5 ml, GE Healthcare, Cat.No:17-5159-01) coupled onto the AKTAprime FPLC system is used to further purify androcam from residual contaminants. The following buffers are used on the Q column:

Low Salt Buffer (A): 25 mM KCl, 2 mM EDTA, 25 mM TrisHCl, 10 mM DTT, pH 7.4

High Salt Buffer (B): 1 M KCl, 2 mM EDTA, 25 mM TrisHCl, 10 mM DTT, pH 7.4

After dialysis of pooled HIC-B4 fractions against water, I loaded the pool slowly (1 ml/min) onto the Q column pre-equilibrated with low salt buffer. The iso-electric point (pI) of androcam is 5.12, hence at pH 7.4 the protein will be negatively charged and readily bind the anion exchanger resin of the Q column. After injection, I washed the Q column with five column volumes of low salt buffer. Androcam is eluted from the Q column by a salt gradient that ramps quickly to 30% Buffer B and then slowly (over ten column volumes) to 100% Buffer B using the automated gradient program shown in Table 2.1. The elution profile and SDS-PAGE of selected fractions from Q column salt gradient are shown in Figure 2.4. The fractions containing androcam are pooled, dialyzed to the appropriate salt/buffer conditions, lyophilized and stored at -20°C to be resuspended later to make an NMR sample.

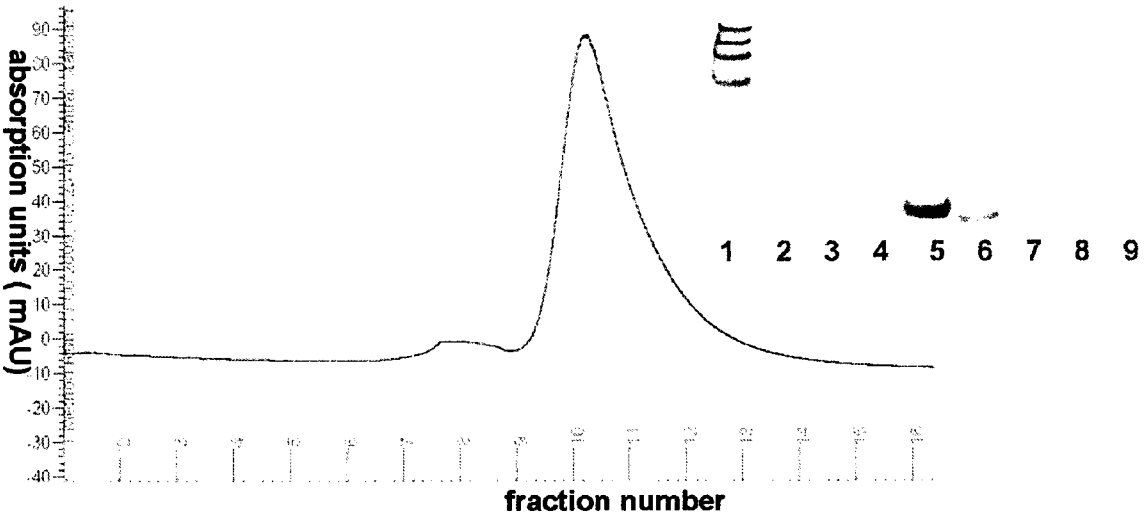


Figure 2.3 Elution profile of Phenyl column 2 from buffer HIC-B4 wash.

Tryptophan 143 of androcam is monitored by absorption at 280 nm. Inset: 15% SDS polyacrylamide gel of HIC-B4 fractions. Lane 1: protein standard, Lanes 2-9: HIC-B4 fractions 7-13. Lanes 4-7 (fractions 9-12) contain androcam.

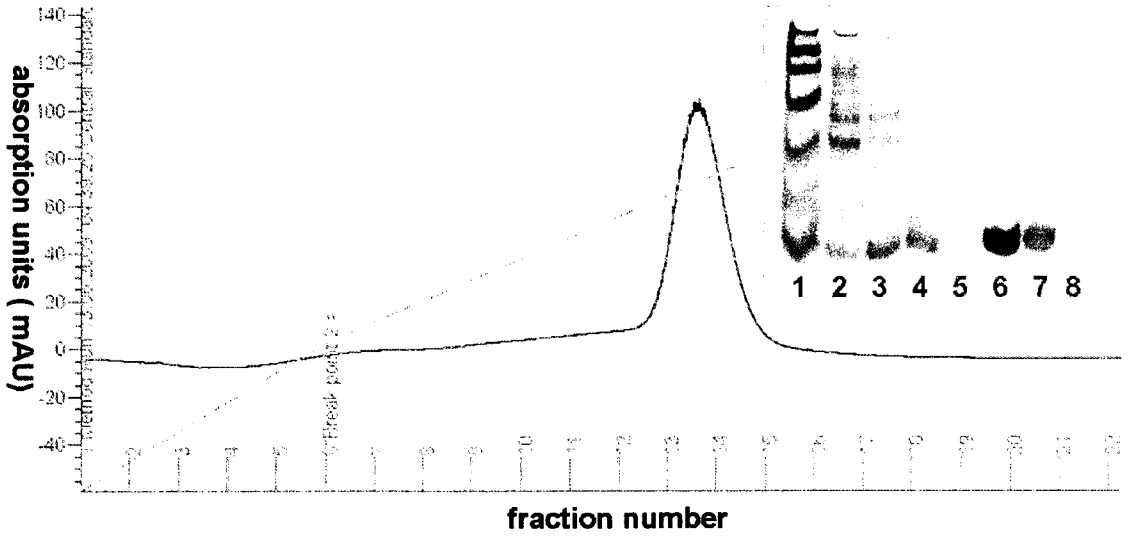


Figure 2.4 Elution profile of salt gradient on Ionic Q column.

Tryptophan 143 of androcam is monitored by absorption at 280 nm. Green line shows break points in the gradient ramp. Inset: 15% SDS PAGE of eluted fractions from salt gradient. Lane 1: protein standard, Lane 2: F/T pellet, Lane 3: F/T supernatant, Lane 4: pooled fractions 7-13 of HIC B4 wash after dialysis to low salt condition, Lanes 5-8: salt gradient fractions 12-15. Lanes 6 and 7 (fractions 13 and 14) contain androcam

Table 2.1 Salt gradient program applied on ion exchange (Q) column.

Break point	Total volume (ml)	% High Salt buffer	Flow rate (ml/min)	Fraction volume (ml)
1	0	0	1	3
2	15	30	1	3
3	64	100	1	3

2.6 Making NMR samples (high calcium)

Fractions from the Q column salt gradient that contain androcam are pooled together and dialyzed against a buffer containing 0.33 mM KCl, 0.33 mM Tris-HCl, 0.33 mM $CaCl_2$ and 0.33 mM DTT. After dialysis the sample is frozen, lyophilized, and then stored at -20°C. To make an NMR sample, the lyophilized powder is resuspended in 315 µl MilliQ water + 35 µl D_2O, the pH is adjusted to 7.4 (neglecting the deuterium isotope effect) and 3.5 µl of freshly prepared 1 M DTT solution is added to make the sample 10 mM in DTT. 0.35µl of 100 mM d_4 TSP is added to the sample as an internal standard for chemical shift referencing.

The dialysis conditions were chosen to generate a 350 µl NMR sample that is 10 mM in Ca^{+2}, making all three sites saturated with Ca^{+2}. With the above purification protocol, I was able to make doubly (^{15}N ^{13}C) or singly (^{15}N) labeled androcam samples at 1 mM concentration or higher that were stable for more than three months, enough to acquire all the NMR data needed for assignment and structure calculations.

NMR sample of androcam in [10 mM Ca^{+2}] (^{15}N ^{13}C labeled):

1.02 mM androcam, 10 mM DTT, 10 mM Tris-HCl, 10 mM KCl, 10mM $CaCl_2$, 0.1 mM d_4 TSP, pH 7.4, 25°C.

NMR sample of androcam in [10 mM Ca^{+2}] (^{15}N labeled):

1.8 mM androcam, 10 mM DTT, 10 mM Tris-HCl, 10 mM KCl, 10 mM $CaCl_2$, 0.1 mM d_4 TSP, pH 7.4, 25°C.

2.7 $^{1}H^{15}N$ HSQC spectrum of androcam

Preliminary 2D proton nitrogen HSQC spectra (e.g. Figure 2.5) with 20 minute acquisition times showed ~145 well resolved peaks with similar intensities, indicating that the protein is well folded and concentrated enough to acquire 3D NMR spectra in reasonable time periods. Each peak in this spectrum represents a backbone or side chain amide proton pair. The position of a peak is indicative of the chemical environment in which the particular residue is present. For example, the three most downfield peaks in proton chemical shift (10.41, 10.54, 10.73) are backbone amides of conserved glycines found in the loop regions of Ca^{+2} binding EF hands; these glycines adopt unusual backbone torsion angles that result in perturbed NMR chemical shifts. Because changes in peak positions reflect changes in structure or local environment, the 2D ^{15}N HSQC spectrum can be used as diagnostic tool to detect conformational changes or binding events.

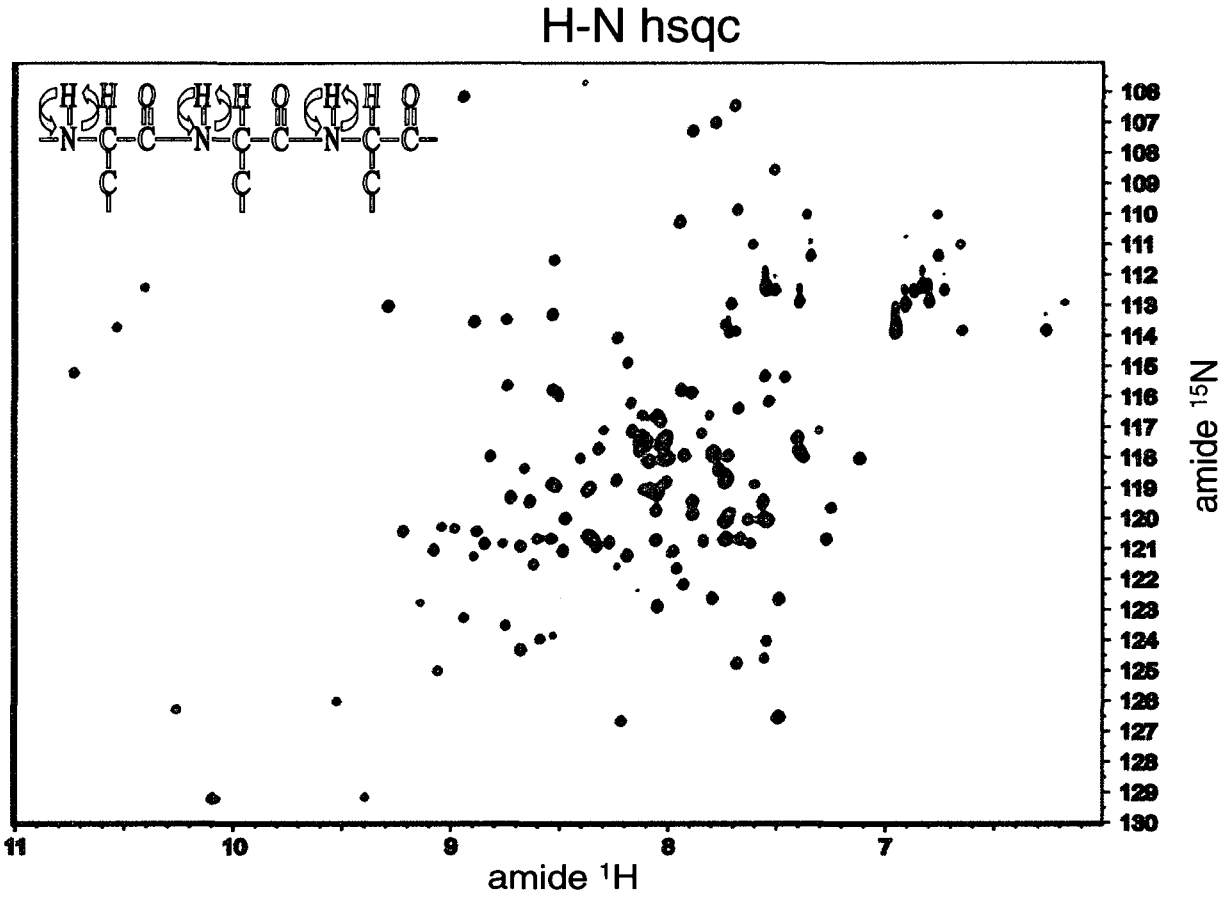


Figure 2.5 $^{1}H^{15}N$ HSQC spectrum of androcam (in 10 mM Ca^{+2})

2D ^{1}H-^{15}N Heteronuclear Single Quantum Correlation Spectrum (HSQC) of androcam. The inset sketch of a typical protein backbone shows the magnetization transfer pathway. Shown on the x axis and Y axes are the proton and nitrogen chemical shifts respectively. Each peak in the spectrum represents an N-H pair of backbone, side chain or indole amides. At the left side of the spectrum are the three glycine amide peaks whose downfield proton shifts make them "finger print" resonances of EF hands; we infer that ACaM forms three EF hands, and so binds 3 Ca^{+2}. Data acquired using a 800 MHz Varian spectrometer on a 1.8 mM double labeled ACaM sample (10 mM KCl, 10 mM Tris-HCl, 10 mM $CaCl_2$, 10 mM DTT, pH 7.4, 25°C).

3. CALCIUM BINDING IN ANDROCAM

3.1 Calcium titration of androcam

It has previously been reported that Ca^{+2} binds to androcam at three sites, two high affinity sites with K_ds of 25 nM and 56 nM and a weak site with a K_d of 80 μM or weaker (Martin et al., 1999). Sequence comparison to calmodulin (Figure 1.7) shows that androcam has two well conserved EF hand motifs in the C lobe, but one pseudo EF hand and one completely non-canonical EF hand in the N lobe. It seems the high affinity sites are the canonical Ca^{+2} binding EF hands 3 and 4 in the C lobe, whereas the weaker site would be EF hand 1 or 2 in the N lobe. I have used NMR spectroscopy to figure out which residues are involved in binding Ca^{+2} at the weak site and accurately determine its affinity (K_d) resolving the uncertainty in the value of 80 μM expressed in their paper by Martin et al, 1999

NMR chemical shifts can be determined accurately and precisely for protein samples at ~100 μM, so binding equilibria with affinities (K_d) in this concentration regime can be determined by titrations using chemical shift changes ($\Delta\delta$). The magnitude of $\Delta\delta$ can also be indicative of the extent and location (site specific or global) of conformational change upon binding. Titrating the weak Ca^{+2} binding site and observing chemical shift changes not only allows us to determine the location of the site (residues that show chemical shift changes) but also accurately determine the binding constant. Titrating the tight sites (K_d = 25 nM, 56 nM) would require protein concentrations in the nM range, which is too dilute to do NMR experiments. Moreover, these sites would be constitutively Ca^{+2} loaded under physiological conditions and their K_ds are accurately

determined by Martin et al. Using the purification protocol described in chapter 2, I prepared a 190 μM androcam NMR sample dialyzed to ~11 μM total calcium as a starting point to ensure complete saturation of the tight sites and at least 96% vacancy (assuming K_d = 80μM) of the weak site. I then titrated it with increasing amounts of $CaCl_2$ in sixteen steps to about 46 mM calcium. 2D ^{15}N HSQC spectra were acquired at each titration point and peak movement monitored to detect the extent and location of conformational change upon Ca^{+2} binding.

3.2 Slow and fast chemical exchange

In solution, the Ca^{+2}-bound and Ca^{+2}-free states are in chemical exchange with each other, and each residue can have distinct chemical shifts in these two states. Figure 3.1 shows how the rate of exchange between the two states influences the observed peak(s) on a single chemical shift axis (Nuclear Magnetic Resonance Spectroscopy by Robert K. Harris).

If the rate of exchange between the two species (R_{ex}) is much smaller than the frequency difference in the chemical shifts of the two species ($\Delta\delta$, in Hz) the species are said to be in 'slow exchange' ($R_{ex} << \Delta\delta$), and each species appears as resolved individual peaks, with the relative intensities of the peaks being proportional to the mole fraction in each state. Conversely, if the rate of exchange between the two species is much greater than their difference in chemical shift ($R_{ex} >> \Delta\delta$), the species are said to be in 'fast exchange', and a single peak appears in the spectrum at a chemical shift determined by the population weighted mean of the shifts of the bound and unbound

states. For intermediate cases, where $R_{ex} \sim \Delta\delta$, the exchange process can cause severe line broadening even in 1D spectra, and in many cases the resonances are not observed.

I titrated the 190 μM androcam sample in [Ca^{+2}] steps of 11 μM, 28 μM, 60 μM, 95 μM, 130 μM, 175 μM, 230 μM, 320 μM, 480 μM, 900 μM, 1.4 mM, 2.0 mM, 2.5 mM, 3.0 mM, 3.7 mM, 5.8 mM, 22.0 mM, and 46 mM. Figure 3.2 shows the superposition of the ^{15}N HSQC spectrum of Figure 2.5 ($[Ca^{+2}]_{total}$ = 10 mM) onto the initial spectrum acquired for the $[Ca^{+2}]_{total}$ = 11μM titration point. Small but significant changes in chemical shift are exhibited by about 40 peaks (~25% of the peaks). Using assignment procedures described in Chapter 4, it was found that most of the peaks that show significant chemical shift changes correspond to amides in the N lobe, consistent with the expectation that Ca^{+2} is binding weakly to this lobe. (A few exceptions include shift changes for residues in the central linker and at the N and C termini; such changes are consistent with non-specific salt effects.) A single peak was observed at each titration point for all peaks that exhibited a chemical shift change between 11 μM and 46 mM, indicating that Ca^{+2} binding to the N lobe is in fast exchange. The binding constant of the N-lobe (K_d = 80 μM or lower) falls in the physiological Ca^{+2} signaling range. Hence, I decided to pursue two structures of androcam; both having a Ca^{+2} loaded C lobe but one with a Ca^{+2} bound and the other with a Ca^{+2} free N lobe.

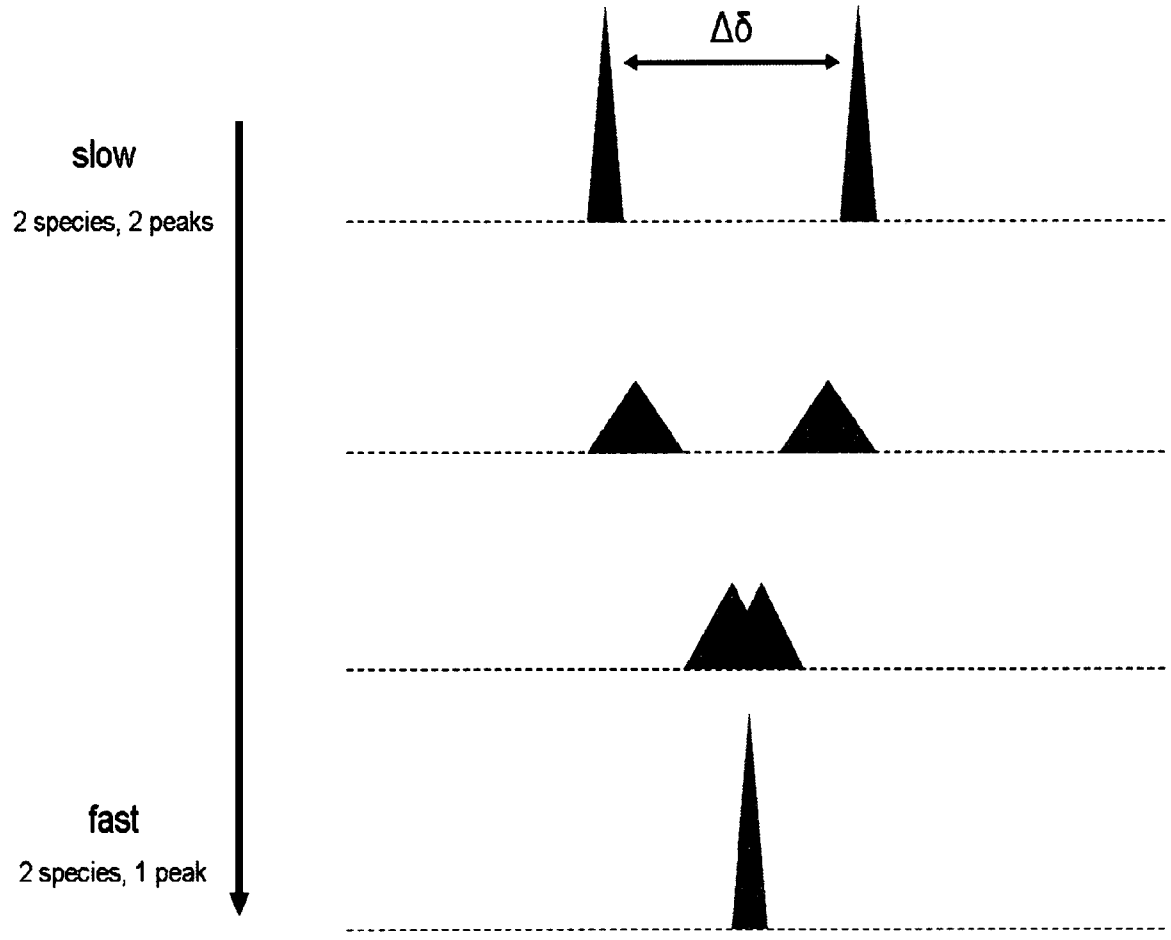


Figure 3.1 "Fast" and "Slow" chemical exchange

The chemical shift position of a given nucleus in each of the two states that are in slow or fast exchange are shown as blue cones. Δδ represents the difference in chemical shift of the nucleus (in Hz) between each state.

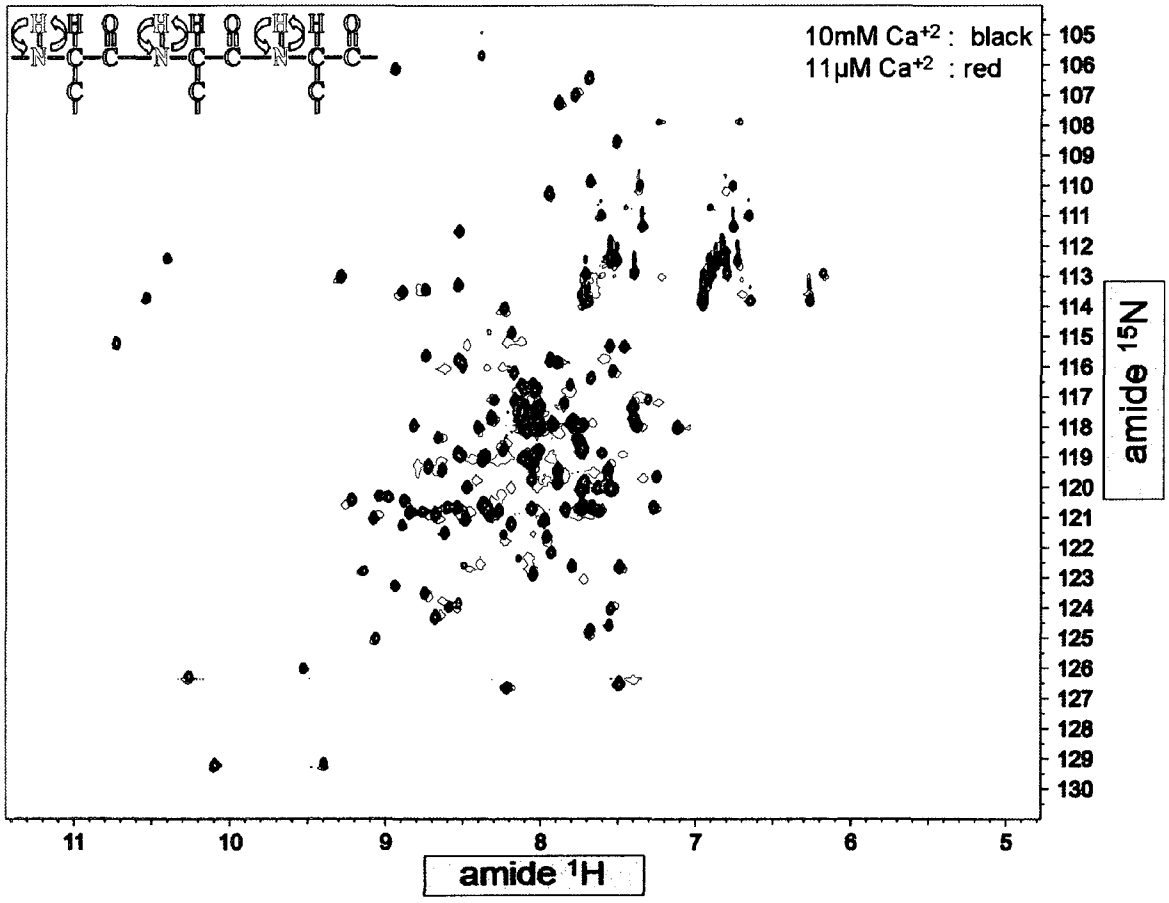
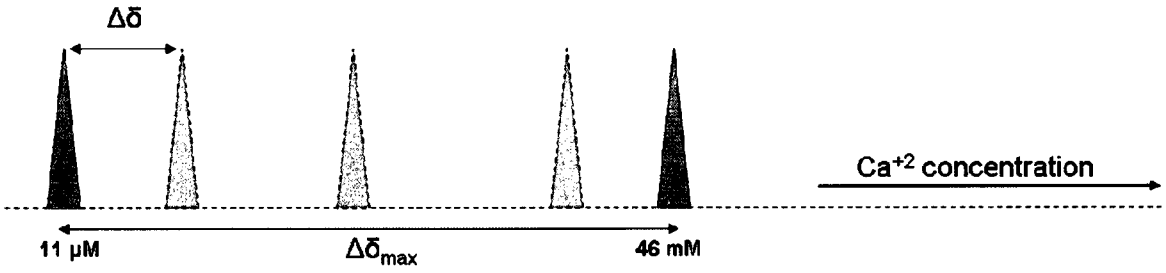


Figure 3.2 Superposition of $^{1}H^{15}N$ HSQC spectra of androcam at 10 mM and 11 μM free $CaCl_2$.

2D ^{15}N Heteronuclear Single Quantum Correlation Spectra (HSQC) of androcam. The inset sketch of a typical protein backbone shows the magnetization transfer pathway. Shown on the X and Y axes are the proton and nitrogen chemical shifts respectively. The spectrum at 11 μM Ca^{+2} (red) is superimposed on the spectrum at 10 mM Ca^{+2} (black) to indicate relative peak movements. Data acquired using a 800 MHz Varian spectrometer on a 190 μM ^{15}N labeled androcam sample (10 mM KCl, 10 mM Tris-Cl, 10 mM DTT, pH 7.4, 25°C).

3.3 Calcium binding constant of the N lobe

The mathematical formulation for the binding of Ca^{+2} to a single site on the N-lobe of androcam is presented in Appendix A. In the regime of fast exchange, the fractional saturation with Ca^{+2} (1-Y) can be determined from the chemical shift changes between bound and unbound states (see schematic, Figure 3.3). Figure 3.4 shows the extent of peak movement upon Ca^{+2} titration from 11 μM to 46 mM for residues L48, D50, A53 and Q62 at selected [Ca^{+2}]. These residues exhibit the largest magnitude change in ^{1}H chemical shift over the course of the titration; G61 also shifts substantially, but predominantly in ^{15}N, not ^{1}H.



$$\Delta\delta_{calc} = \Delta\delta_{\max}\left\{1 - \left[\frac{(A_T - C_T - K_D) \pm \sqrt{(C_T - A_T + K_D)^2 + 4A_T K_D}}{2A_T}\right]\right\}$$

Figure 3.3 In fast exchange, peak position can be related to fractional saturation.

Change in chemical shift for an amide peak in fast exchange between bound and unbound states from 11 μM to 46 mM free calcium. The total chemical shift change is given as $\Delta\delta_{max}$. The fractional change ($\Delta\delta$ / $\Delta\delta_{max}$) is related to the extent of binding as shown in the equation. A_T : total concentration of androcam; K_D : binding constant for a Ca^{+2} ion; C_T : total concentration of calcium.

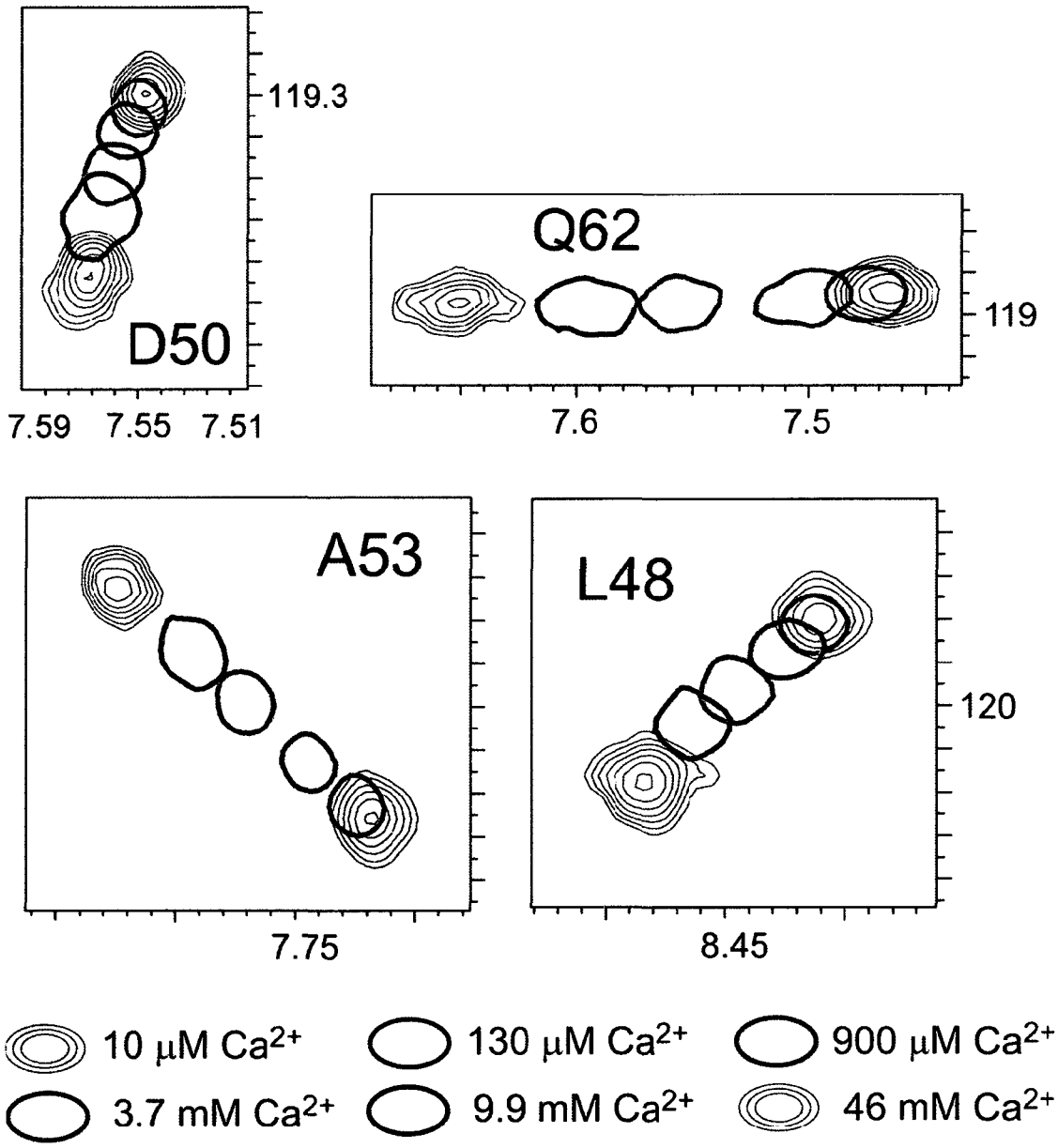


Figure 3.4 Chemical shift changes of selected residues of androcam with [$CaCl_2$]

Individual peak movement in selected 2D ^{15}N HSQC spectra of androcam acquired at 11 μM, 130 μM, 900 μM, 3.7 mM, 9.9 mM and 46 mM $CaCl_2$. Panels show zoom in view of the superimposed spectra around the residues L48, D50, A53 and Q62. X and Y axes show the proton and nitrogen chemical shifts respectively and are drawn to scale. All peak contours are shown at 11 μM and 46 mM [Ca^{+2}]. For clarity, only the lowest contour is shown at 130 μM, 900 μM, 3.7 mM and 9.9 mM. All spectra are contoured to the same level above the noise.

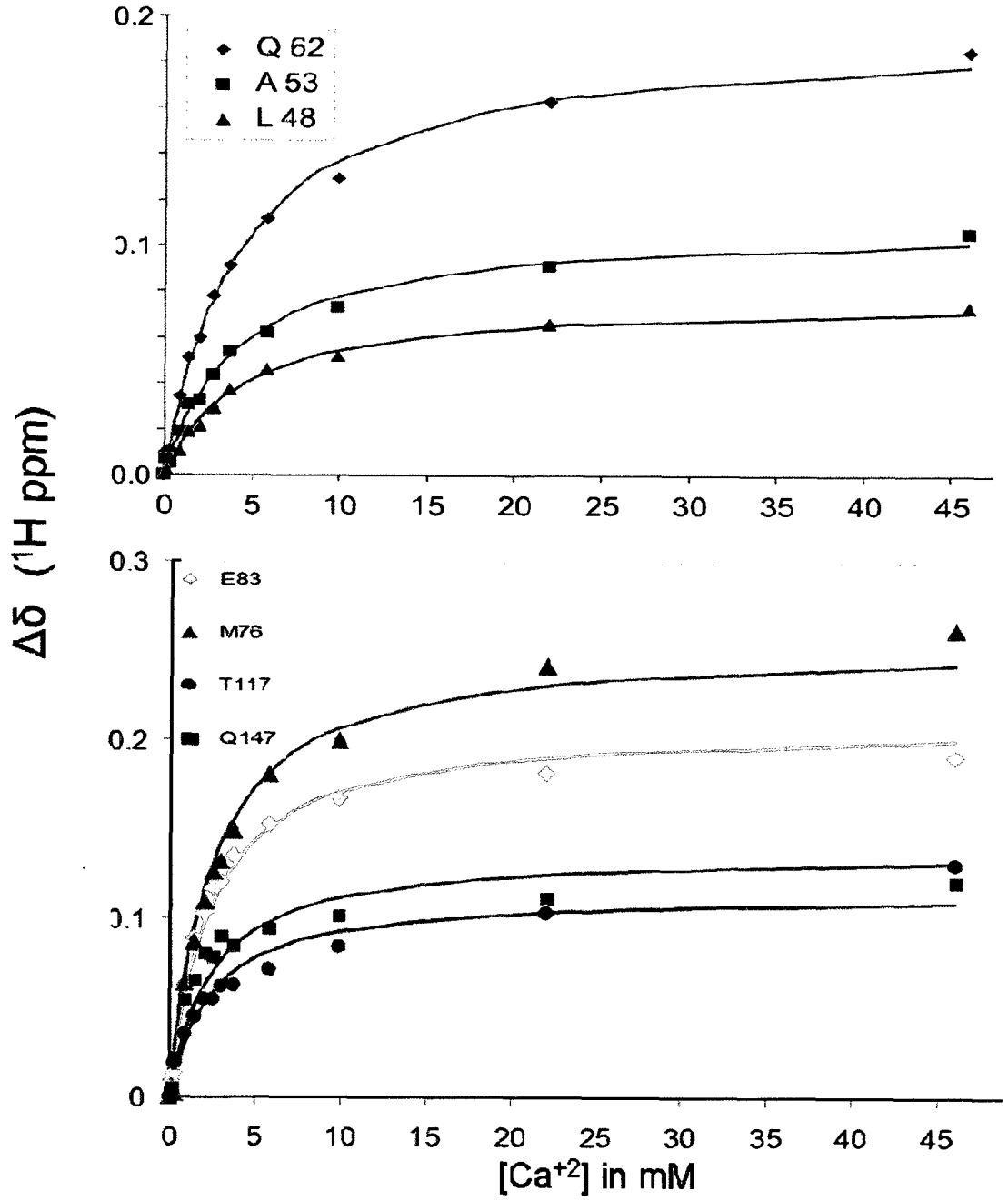


Figure 3.5.A Calcium titration curves for selected residues of androcam

The change in chemical shift of the amide proton (on Y axis) of selected residues with change in Ca^{+2} concentration (on X axis). Markings indicate actual data points; smooth lines represent the best fit through the data set for each residue. Top Panel: N lobe residues like L48, A53 and Q62 fit a two state binding curve (K_D = ~4.1 mM) (χ^2 = 0.0055). Bottom Panel: Residues near the central linker (E83, M76), in the C-lobe (T117) and at the C terminus (Q147) give poor fit (χ^2 = 0.0212) to a binding curve.

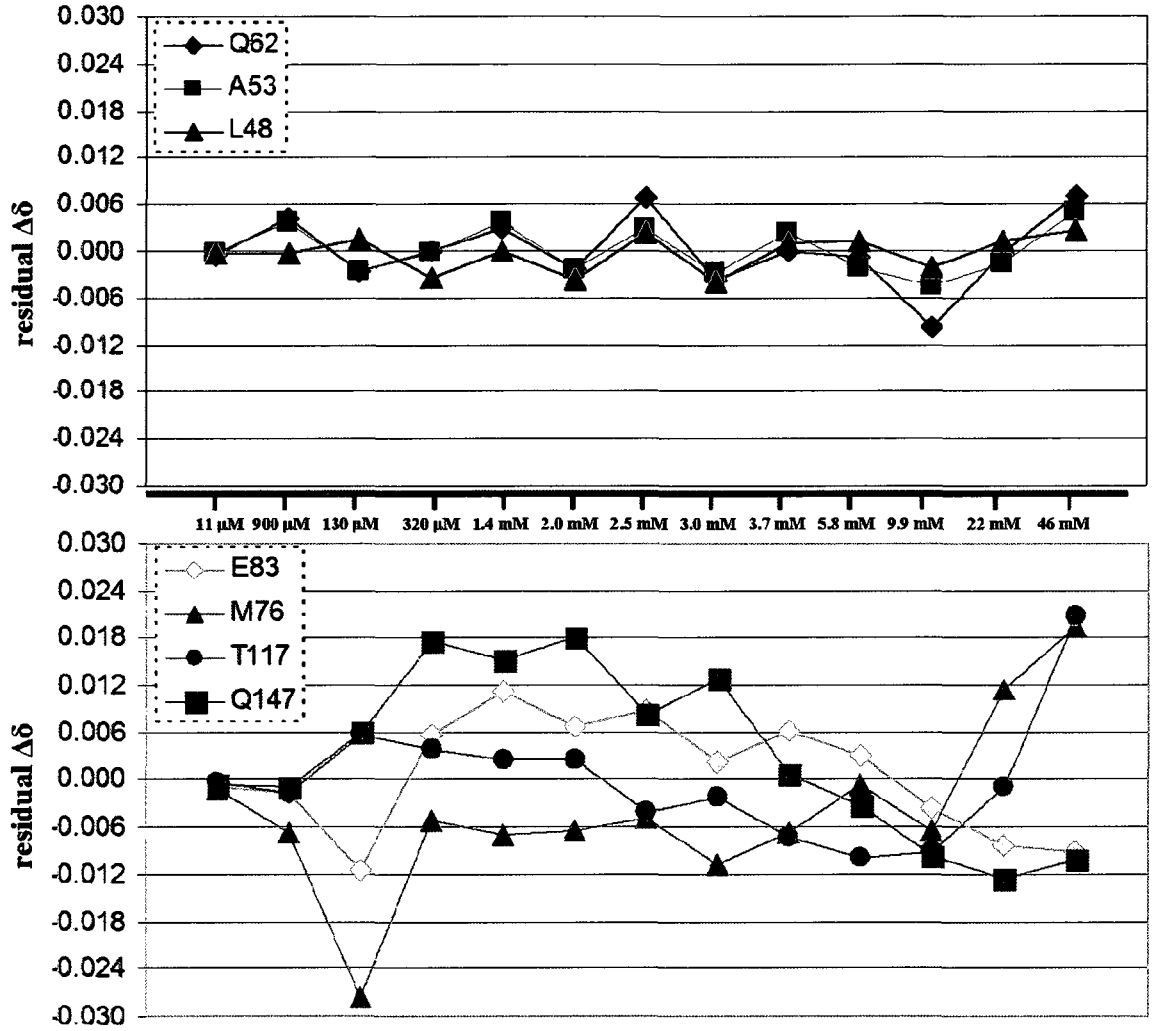


Figure 3.5.B Residual chemical shifts on calcium titration curves for selected residues of androcam

The difference in chemical shift of the actual data from the theoretical fit curve (residual Δδ) for amide proton (on Y axis) of selected residues with change in Ca^{+2} concentration (on X axis). Central horizontal bar with markings indicate total calcium concentrations at each data point. Residual Δδ for N lobe residues L48, A53 and Q62 (upper panel) are smaller and random compared to those for residues near the central linker (E83, M76), in the C-lobe (T117) and at the C terminus (Q147) (lower panel) which are large and systematic.

Figure 3.5.A shows the change in amide proton chemical shift at each titration point plotted against [Ca^{+2}] for selected residues of androcam. For N lobe residues like L48, A53 and Q62 the data points match to a theoretical binding curve with a K_D = ~4.1 mM determined by simultaneously fitting all three curves (χ^2 = 0.0055). Linker and other C lobe residues like M76, E83, T117 and Q147 fit poorly (χ^2 = 0.0212) to any binding curve. Thus the N lobe peak movements correspond to a Ca^{+2} binding event with an affinity of 4.1 mM, or fifty-fold weaker than the affinity of 80 μM determined by (Martin et al., 1999). From Eqn. (7) of appendix A.1

$$Y = \frac{A_T - C_T - K_D \pm \sqrt{(C_T - A_T + K_D)^2 + 4A_T K_D}}{2A_T} \qquad \text{..... (7).}$$

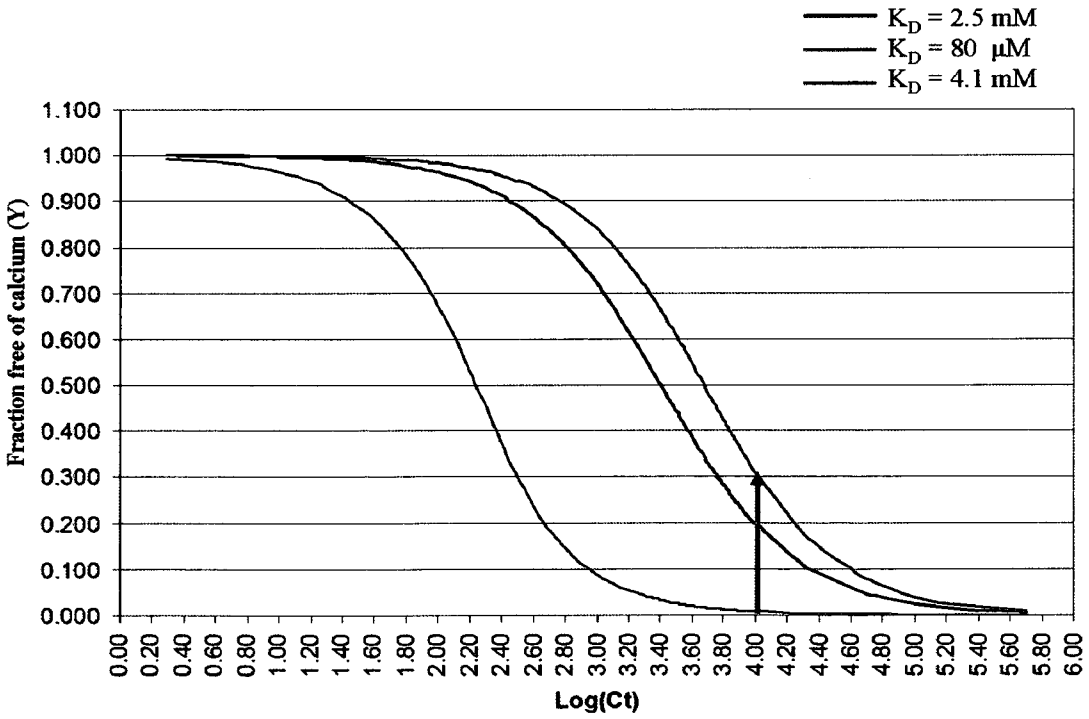


Figure 3.6 Fractional saturation of the N lobe with [Ca^{+2}]

For mathematical derivation of Y = f(C_T) refer to appendix A.1. Fraction of protein free of Ca^{+2} (Y) in the N-lobe is plotted against Log of total protein in excess of a saturated C-lobe (C_T in μM). Curves represent three different binding constants (K_D = 80 μM, 2.5 mM and 4.1 mM) for a total androcam concentration of A_T = 1.5 mM. Red arrow shows that at 10mM [Ca^{+2}], Y=0.3 i.e. only 70% of the N-lobe is saturated with calcium

From Figure 3.6, it can be seen that the N-lobe will be fully saturated with [Ca^{+2}] at ~1 M $CaCl_2$, a highly non-physiological concentration. NMR spectroscopy is not feasible in 1 M salt solution; hence the structure of fully calcium-loaded androcam will not be pursued. Even above 10 mM $CaCl_2$, the pulse widths become exceedingly long (^1H pulse width is >10 μs) or power levels must rise well above normal (^1H pulse power >57 dB). Pulsing the spectrometer with high power for long times can be very detrimental to the probe. However, the structure of androcam in low calcium (~10 μM) with a Ca^{+2} saturated C lobe and Ca^{+2} free N-lobe, which I term 'apoN' androcam, can be determined by NMR spectroscopy. I also decided to pursue a partially Ca^{+2} saturated N lobe structure in as much $CaCl_2$ as possible. This is done to determine the extent of conformational changes in the N-lobe upon Ca^{+2} binding and to see if any effects occur on the already saturated C-lobe structure. I chose 10 mM $CaCl_2$ as the 'high calcium' condition: this should result in the N-lobe being about 70% Ca^{+2} loaded (Figure 3.6), and NMR spectra give high signal/noise ratios with good peak dispersion and moderate pulse widths appropriate for extended data collection. Since the N-lobe is in fast exchange, the structure solved at 10mM [Ca^{+2}] will be a conformational average of the 70% bound state and the 30% free state. A comparison of the two structures would be indicative of the direction and extent of conformational change upon Ca^{+2} binding to the N lobe. The 10 mM $CaCl_2$ samples are prepared as already described in Section 2.6 and the strategy to obtain an apoN sample is discussed in Section 3.5.

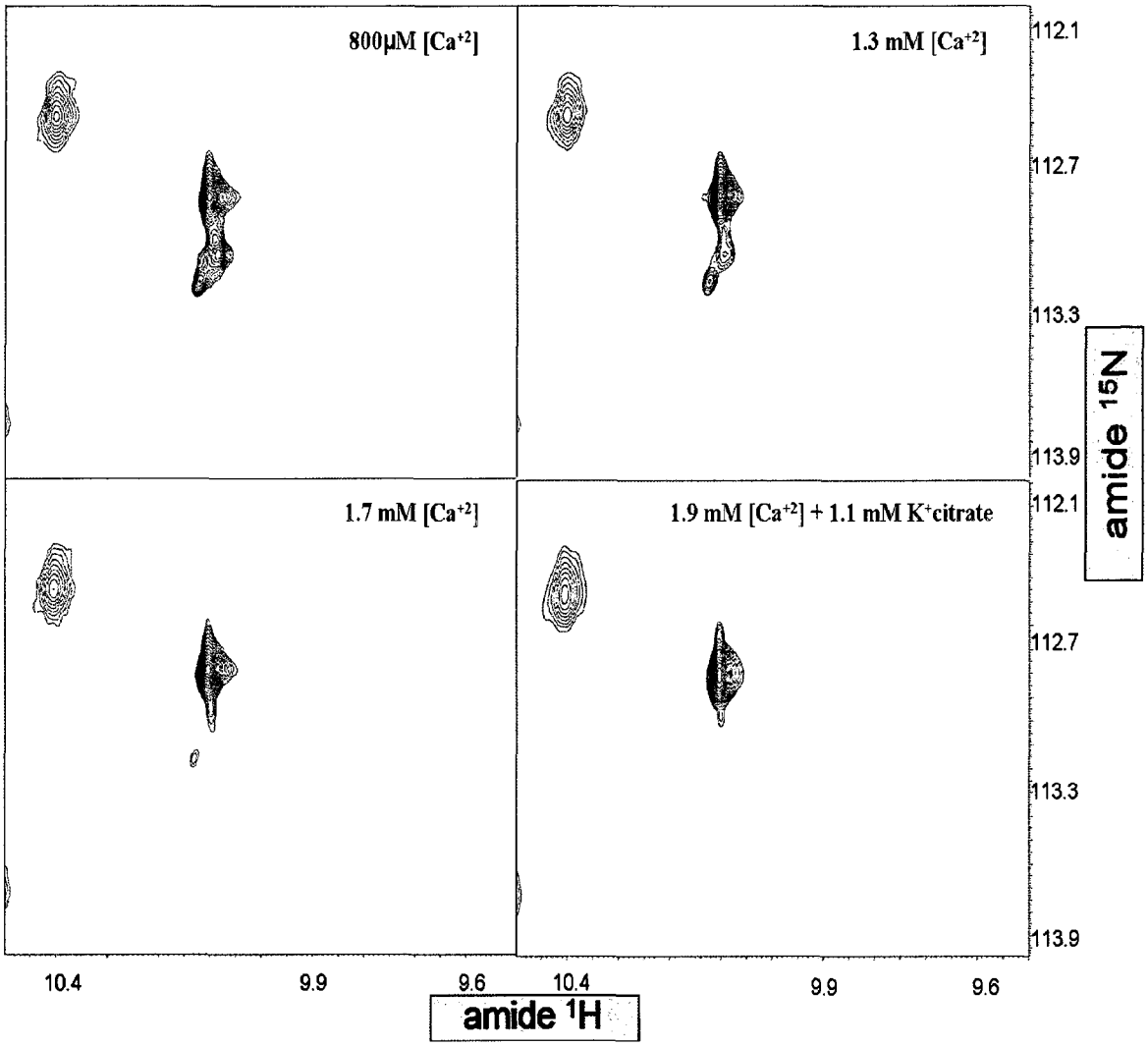


Figure 3.7 Amide peaks of androcam C-lobe show slow exchange with Ca^{+2}

Panels show zoom in view of the W143 indole NH peak in 2D ^{15}N HSQC spectra of androcam acquired at different calcium and/or buffer concentrations. All spectra are contoured to the same level above the noise. X and Y axes show the proton and nitrogen chemical shifts respectively and drawn to scale. Sample contains 1.8 mM ^{15}N labeled androcam, 10 mM Tris-HCl, 10 mM KCl, 5 mM DTT. Free calcium and the total buffer (potassium citrate K_D = 215 μM) concentrations are shown in top-right of each panel. All well resolved C lobe peaks show similar behavior and extent of splitting.

3.4 The androcam C lobe binds calcium in slow exchange

If dialyzed for more than 36 hours under low [Ca^{+2}] conditions (~2 μM) and at very low (μM) salt, the C lobe of androcam begins to lose calcium. Under these conditions, the amide peaks of the C-lobe give two to four distinct peaks: one for the unbound state, one for the fully saturated state, and sometimes distinct peaks are seen for site 3 only or site 4 only being occupied. This indicates that binding of calcium to the C-lobe is in slow exchange. The four panels in Figure 3.7 show the effects of increasing [Ca^{+2}] on the indole NH peak of Tryptophan 143. At low [Ca^{+2}], the W143 indole amide peak shows multiple peaks arising from slow exchange. With increasing [Ca^{+2}], the C-lobe gets more and more occupied, showing reduced intensity for the unbound peak and finally only the bound peak prevails at complete saturation.

3.5 NMR sample preparation for "apoN" androcam

Androcam is expressed and purified on Phenyl and Q columns as described previously in Sections 2.1-2.5. To prepare an 'apoN' NMR sample, Q column fractions that contain androcam are pooled and dialyzed to 2 mM Tris-HCl, 2 mM KCl, 0.438 mM DTT and 1.75 mM $CaCl_2$. After dialysis for 24 hours with buffer changed every 8 hours, the sample volume is reduced to 1.75 ml using an Amicon Ultra centrifugal filter (Cat: UFC800308). 500 μl of retentate is made 5 mM in DTT and a 2D N-HSQC diagnostic spectrum is acquired to check if dialysis has led to apoN conditions. The entire retentate is lyophilized and stored in powder form at -20°C. Hydrating the lyophilized powder to make an NMR sample effectively concentrates androcam and calcium ~5 fold relative to

diagnostic spectrum. The 2D N-HSQC is re-acquired to ensure apoN conditions prevail under concentrated conditions. Next, a calcium chelator is added to the sample and another 2D HSQC is acquired. If no peak movement is seen, we can confirm that 'apoN' conditions are met. The derivation of the mathematical equations for fractional saturation of the N-lobe is presented in Appendix A.2. The chelator must have a binding affinity for Ca^{+2} that is stronger than the N-lobe (K_D = 4.1 mM) but weaker then the C-lobe (K_Ds = 56 nM, 25 nM) to ensure that the N-lobe remains completely free of Ca^{+2} without stripping any Ca^{+2} from the C-lobe. Potassium citrate with K_D = 215 μM for Ca^{+2} (Rechnitz, 1969) fits the requirement. To make the NMR sample, the lyophilized powder is re-suspended in 300μl H_2O + 50μl D_2O + 3.5 μl 1 M DTT + 2.5 μl of 140 mM potassium citrate. 0.35 μl of 100 mM d_4 TSP is added to the sample as an internal standard for chemical shift referencing.

With the above protocol, I was able to make doubly (^{15}N ^{13}C) or singly (^{15}N) labeled 'apoN' androcam samples at 1 mM concentration or higher that were stable for more than three months, enough to acquire all the NMR data needed for assignment and structure calculations.

<u>NMR sample of apoN androcam (^{15}N ^{13}C labeled)</u>:

1.3 mM androcam, 10 mM DTT, 10 mM Tris-HCl, 10 mM KCl, 0.1 mM d_4 TSP, 1 mM potassium citrate, pH 7.4, 25°C.

<u>NMR sample of apoN androcam (^{15}N labeled)</u>:

1.8 mM androcam, 10 mM DTT, 10 mM Tris-HCl, 10 mM KCl, 0.1 mM d_4 TSP, 1 mM potassium citrate, pH 7.4, 25°C.

4. NMR DATA ACQUISITION, ANALYSIS, AND INTERPRETATION

4.1 Overview

Some elements found in macromolecules have naturally abundant isotopes, such as ^{1}H and ^{31}P, whose properties as 'spin ½ nuclei' can be readily exploited for NMR studies. Other elements have abundant isotopes that lack nuclear spin (^{12}C, ^{16}O) or have unfavorable properties for solution NMR studies (^{14}N), but some stable isotopes of these elements, especially ^{15}N and ^{13}C, have nuclear spin quantum number of ½ and are well suited to NMR studies. When solving the solution structure of a protein of more than 100 residues by NMR spectroscopy, it is highly advantageous to express the protein in isotope enriched minimal media so that every nitrogen nucleus in the molecule is ^{15}N and every carbon nucleus is ^{13}C. The ^{1}H, ^{15}N and ^{13}C nuclei of backbone and side chain atoms of a well-folded protein exhibit a range of chemical shifts that depend upon electronic environment (such as hydrogen bonding) and local conformation (backbone and side chain torsion angles. Uniform (^{15}N, ^{13}C) labeling permits the application of a suite of triple-resonance (^{1}H, ^{13}C, ^{15}N) experiments that exploit strong homo- and hetero-nuclear one bond J couplings to correlate resonances with one another so that the resonance frequencies of essentially every ^{1}H, ^{15}N, and ^{13}C in a protein can be resolved and identified (Bax et al., 1994b)

The first step in NMR structure determination of a labeled protein is therefore the acquisition of 2D and 3D spectra that correlate ^{1}H, ^{15}N, and ^{13}C resonances using through-bond J couplings. These spectra fall into two general classes: one set of

experiments establishes intra- and inter-residue correlations among resonances of backbone atoms, and a second set establishes intra-residue correlations among backbone and sidechain atoms of each residue. Using these spectra, one establishes the chemical shift of each nucleus, and especially each ^{1}H, in the protein. Next one measures Nuclear Overhauser Effects (NOEs), which are relaxation events that occur between two proton nuclei because of dipolar interactions through space. The intensity of an NOE is inversely proportional to the sixth power of the distance separating the two nuclei, and so the intensity of cross peaks in NOESY spectra can give semi-quantitative indications of the relative separations in space among different hydrogens. The distance information from these NOESY spectra is often sufficient to specify the structure of the protein, but other NMR data such as J coupling data are often used as supplements to the NOE data. Three-bond J couplings (^{3}J) are sensitive to the torsion angle between the atoms, and thus can be used to specify backbone or side chain torsion angles; the ^{3}J data themselves can be used directly, or structural inferences (*trans* vs. *gauche*) can be drawn and used in the structure determination protocol. Other restraints that can be inferred from experimental data or from preliminary structures, such as amide hydrogen to carbonyl oxygen hydrogen bonding restraints in helices or sheets, can also be parameterized and incorporated into the structure determination. The process of combining the experimental data to yield a three-dimensional structure is formulated as a minimization problem, in which the goal is to minimize differences between the protein model and the experimental restraints while maintaining good geometry for the protein conformation. In practice, this search is usually undertaken using simulated annealing: molecular dynamics is used to sample conformational space and seek a global energy minimum for the experimental restraints

expressed as pseudo-energy functions together with energy expressions for protein bond lengths, bond angles, torsion angles, and non-bonded interactions. This procedure can be biased away from true structures if NOE distance restraints are incorrectly assigned (or if other structural data are in error), and such biases are not always easy to detect.

In this work, I have input the distance restraint data and all other experimental restraints into the program ARIA (Ambiguous Restraints for Iterative Assignment) (Linge et al., 2003). ARIA uses the set of chemical shifts that I determined from 2D and 3D spectra plus a partially assigned list of NOE cross peaks and alternately performs (1) a molecular dynamics simulated annealing search in CNS (Crystallography and NMR System) that identifies low energy structures consistent with the data and (2) a structure-based and chi-squared evaluated identification of the atom pairs that give rise to NOE cross peaks. ARIA begins with a few unambiguous NOE assignments (those NOE cross peaks whose chemical shifts are unique) plus a great many ambiguous NOE peaks and calculates a set of preliminary structures that is consistent with all of these restraints. ARIA uses these preliminary structures to resolve some of the ambiguities in the NOE assignment list, and then calculates another set of structures. In this way, ARIA iteratively bootstraps its way to a final set of NOESY peak assignments and structures. ARIA identifies any experimental data that are inconsistent with the current round of structures and flags them for inspection by the user, facilitating and automating the process of assigning NOE cross peaks and ensuring that human bias and error is minimized. The remainder of this chapter describes the NMR data I acquired and how they have been converted into data or restraint files for ARIA; the structure determination itself and analysis of the structures are presented in Chapter 5.

4.2 Chemical shift assignments

In addition to the ^{15}N HSQC spectrum discussed in Section 2.7, other 2D and 3D data are acquired to assign the chemical shifts of all ^{1}H, ^{15}N and ^{13}C nuclei. Use of 3D NMR data help resolve overlapping 2D peaks and provide additional chemical shifts e.g. HNCO and HNCACB.

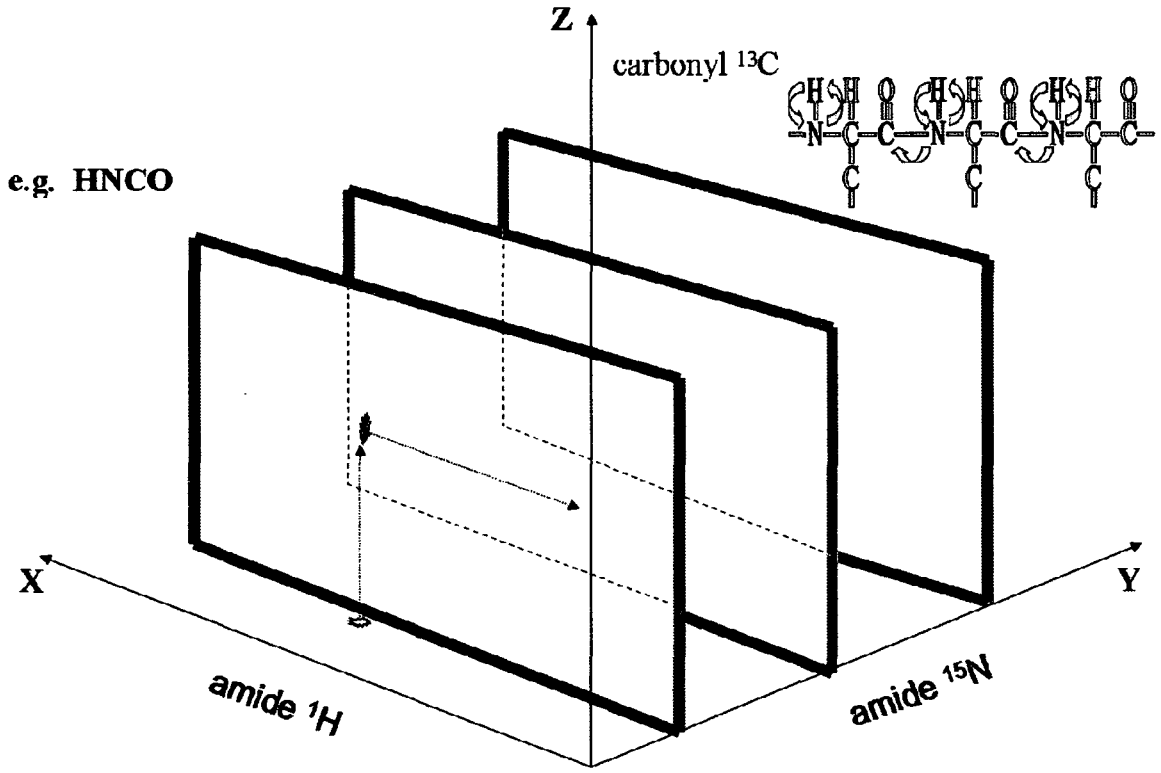


Figure 4.1 Schematic drawing of a 3D NMR spectrum. e.g. HNCO

Cartoon representation of a 3D HNCO spectrum. The amide ^{1}H and amide ^{15}N of a HSQC are shown along X and Y axes respectively. HNCO resolves every amide N-H peak (except the 1st residue) in the XY plane, into the chemical shift of the carbonyl carbon (C') of the preceding residue along the Z axis. Inset shows the magnetization transfer pathway along the protein backbone (Ikura et al., 1990b).

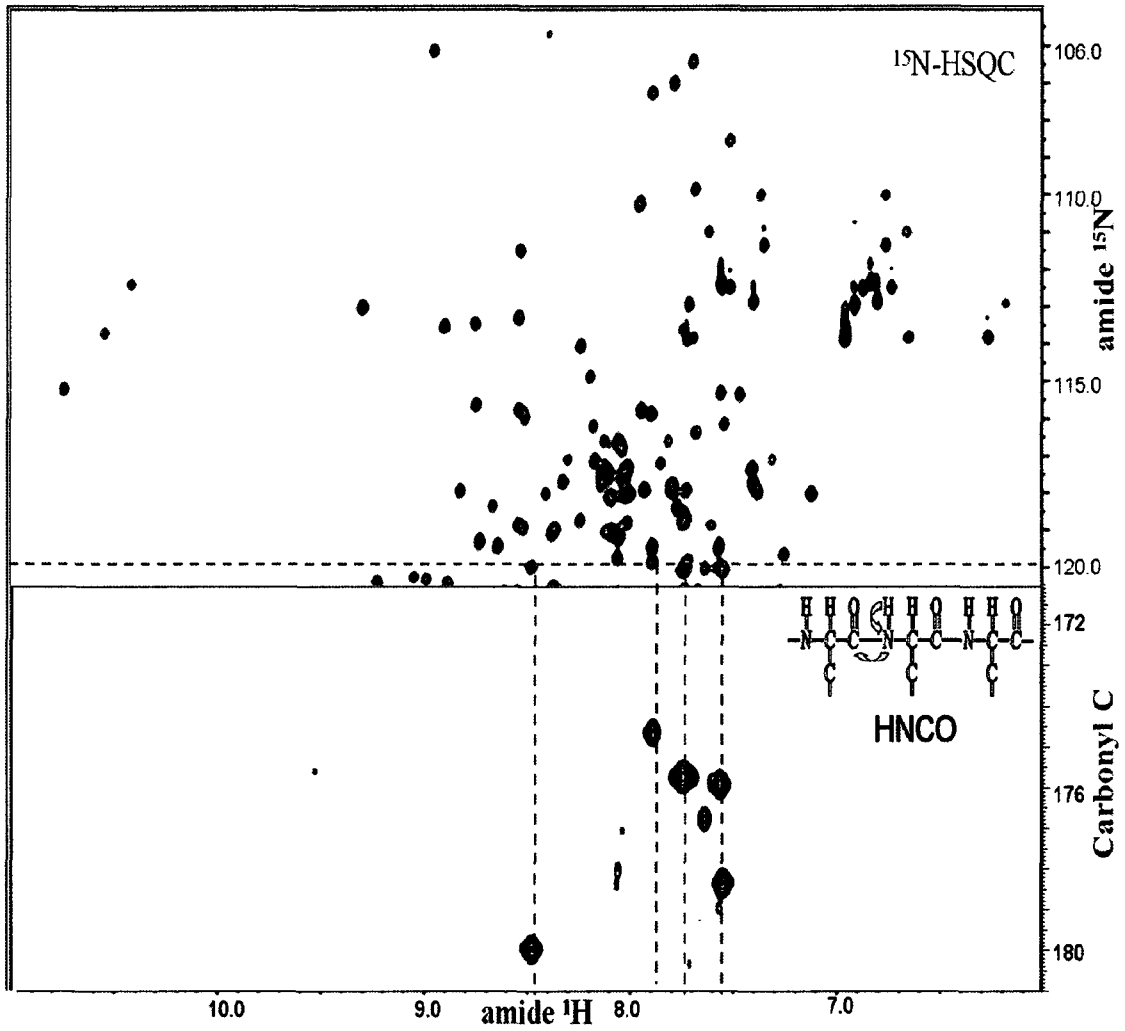


Figure 4.2 3D HNCO spectrum correlates each amide (^{1}H, ^{15}N) chemical shift pair to the carbonyl ^{13}C chemical shift of the preceding residue

Dotted red line represents a horizontal slice at any particular amide N chemical shift. Inset shows the magnetization transfer pathway.
Top panel: partial view of ^{15}N HSQC of androcam above the horizontal slice.
Bottom panel: the amide ^{1}H:carbonyl C' plane from the 3D HNCO spectrum of androcam; taken at the ^{15}N chemical shift of dotted red line. C' peak of the preceding residues are shown by vertical blue lines. Overlapping peaks at ^{1}H,^{15}N ≡ (7.55,119.9) that could not be resolved in two dimensions are resolved into two C' peaks in the third dimension.

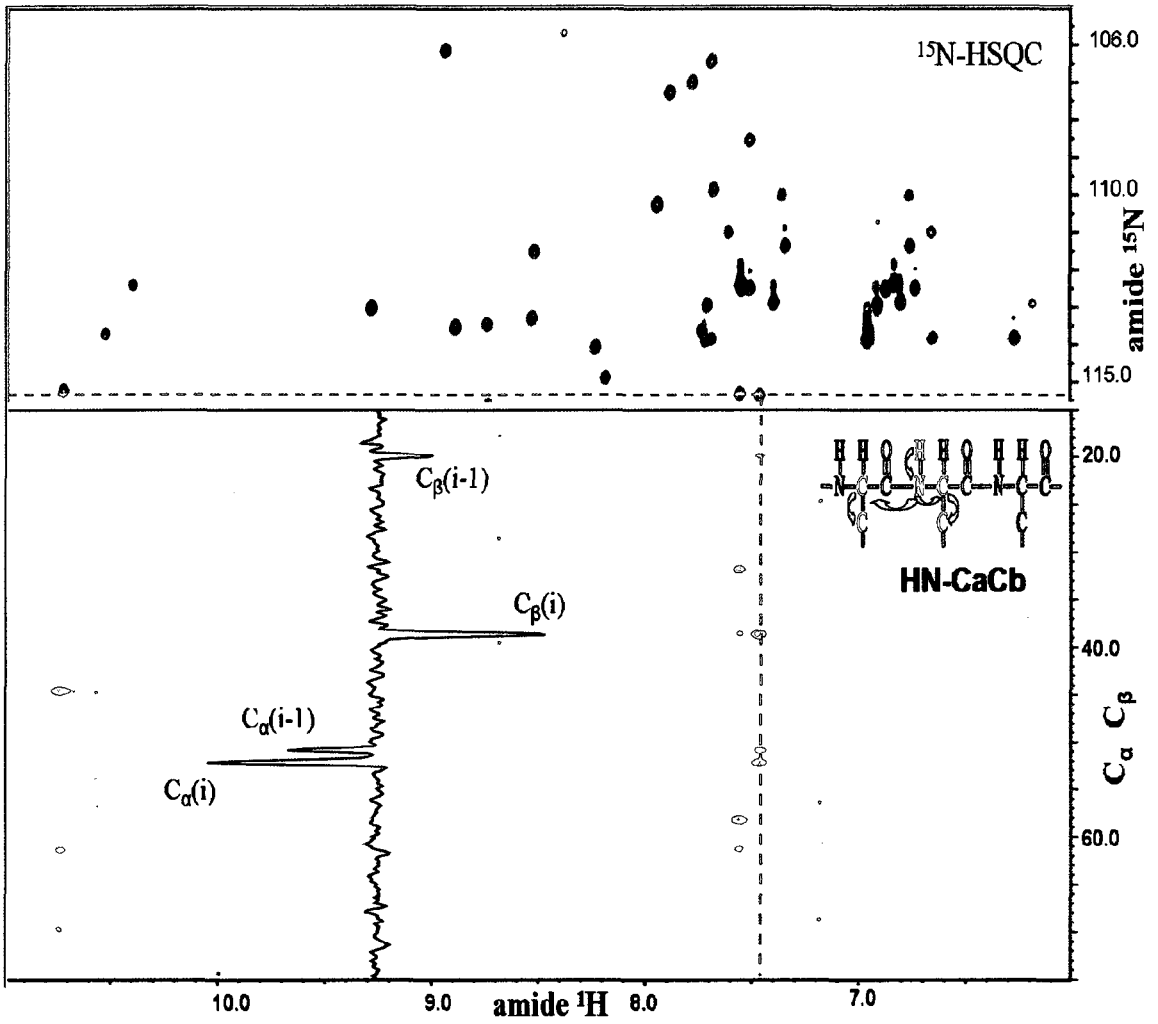


Figure 4.3 3D HNCACB spectrum correlates each amide (^{1}H, ^{15}N) chemical shift pair to the C_α and C_β chemical shifts of its residue and the preceding residue

Dotted red line represents a horizontal slice at any particular amide N chemical shift. Inset shows the magnetization transfer pathway.
Top panel: partial view of ^{15}N HSQC of androcam above the horizontal slice.
Bottom panel: the amide ^{1}H:(C_α,C_β) plane from the 3D HNCACB spectrum of androcam; taken at the ^{15}N chemical shift of dotted red line. The C_α chemical shifts of the current and preceding residue are shown by positive peaks (blue) of higher and lower intensities respectively. The C_β chemical shifts of the current and preceding residue are shown by negative peaks (red) of higher and lower intensities respectively.

4.2.1 Backbone Sequence Walk

The chemical shift assignment process begins with a backbone sequence walk. A series of 2D and 3D NMR data are acquired that correlate different sets of intra- and inter-residue chemical shifts. For example, the HNCO experiment correlates the amide proton and nitrogen shifts of one residue with the carbonyl carbon chemical shift of the previous residue, and the HNCACB experiment correlates the amide proton and nitrogen shifts of one residue with the alpha and beta carbons of that residue and of the previous residue. The task is to record all existing correlations and then decipher the identities, or order, of the amide protons and nitrogens to which the carbonyls, alpha carbons, and beta carbons are correlated. As an illustration of a portion of a sequence walk, consider the following three 2D ^{15}N-HSQC peaks and their chemical shifts from the 3D HNCACB spectrum (10 mM Ca^{+2} sample),

peak # 28	**peak # 137**	**peak # 24**
H^N = 7.798	H^N = 8.026	H^N = 7.931
N = 122.62	N = 117.51	N = 122.17
$C_{\alpha(i)}$ = 54.46	$C_{\alpha(i)}$ = 57.88	$C_{\alpha(i)}$ = 52.966
$C_{\alpha(i-1)}$ = 63.68	$C_{\alpha(i-1)}$ = 54.46	$C_{\alpha(i-1)}$ = 57.88
$C_{\beta(i)}$ = 17.45	$C_{\beta(i)}$ = 28.49	$C_{\beta(i)}$ = 17.91
$C_{\beta(i-1)}$ = 37.65	$C_{\beta(i-1)}$ = 17.46	$C_{\beta(i-1)}$ = 28.53

Peaks in this 3D spectrum lie in columns at the (^{1}H, ^{15}N) shifts of the ^{1}H-^{15}N HSQC spectrum, and so are grouped and initially numbered according to their (^{1}H, ^{15}N) positions for record-keeping purposes. Comparing the C_α and C_β chemical shifts of the (*i*) and (*i*-1) correlations, peaks 28, 137 and 24 can be assigned to amides in succession

along the protein backbone (unless through chemical shift redundancy another set of peaks agrees in a similar way). Knowing that a C_β chemical shift of ~ 17 ppm is unique to alanine (the only residue type with a C_β methyl) and that the androcam amino acid sequence contains only one instance of A-X-A (A53-E54-A55), peaks 28, 137, and 24 can be assigned to residues A53, E54 and A55 respectively. In this fashion, sets of ^{1}H-^{15}N peaks can be ordered and mapped to the primary sequence until every backbone amide is assigned and the corresponding backbone ^{13}C chemical shifts are known for each residue. Because redundancies in chemical shifts complicate this process, I used seven different 3D NMR spectra (CBCA(CO)NNH (Grzesiek and Bax, 1992), HNCA (Ikura et al., 1990a), HNCACB (Ikura et al., 1990a), HCAN (Powers et al., 1991), HNCACO (Yamazaki et al., 1994), HCACO (Grzesiek and Bax, 1993) and HNCO (Ikura et al., 1990a)) to assign the backbone ^{1}H, ^{13}C and ^{15}N chemical shifts for both the 'holo' and 'apoN' androcam samples. Combining the information from these experiments allowed me to unambiguously identify every backbone H^N, N, C', C_α, and C_β chemical shift in these two molecules. I also acquired variants of the 2D ^{13}C-HSQC (for $C\alpha H\alpha$, methyl CH_3 and aromatic CH) resonances.

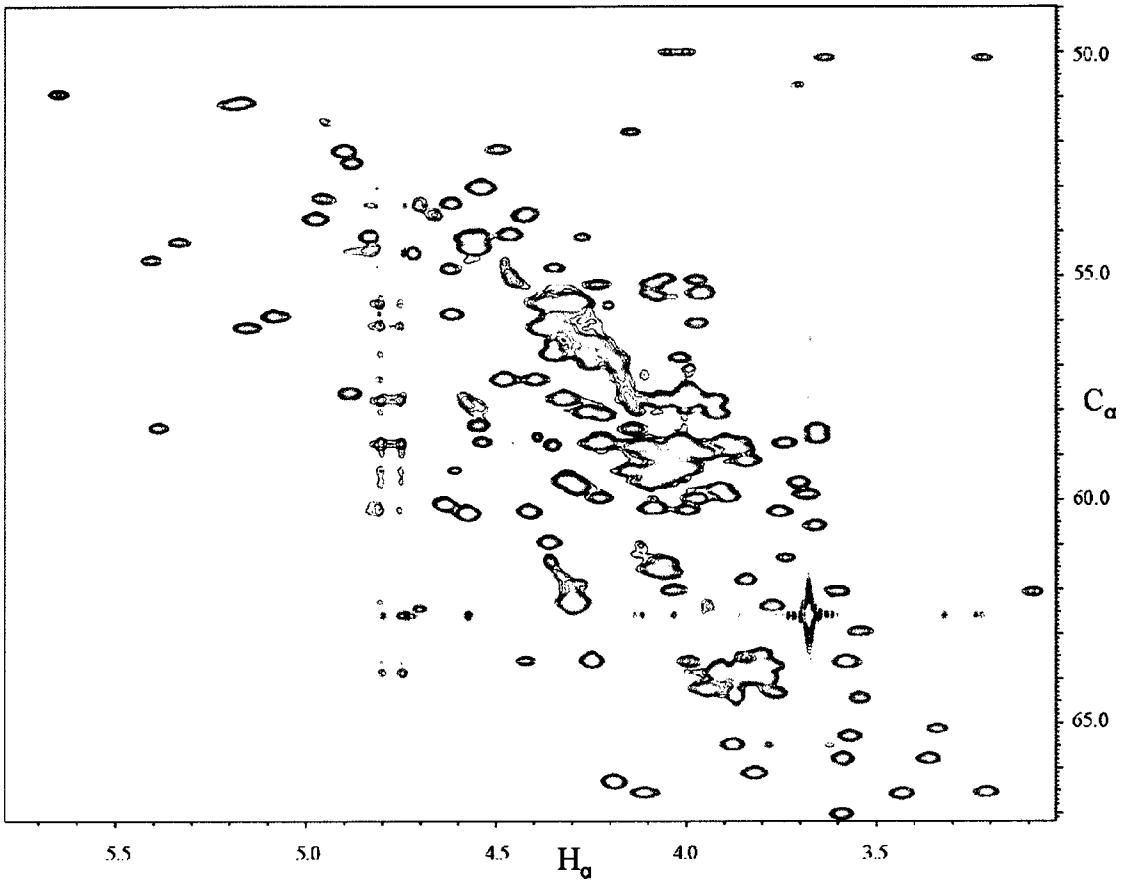


Figure 4.4 2D ^{13}C HSQC of apoN-androcam (Cα region)

Cα region of 1/J ^{13}C-HSQC shows CH pairs with different signs depending on the number of aliphatic $^{1}J_{CC}$ coupling partners for the carbon in question: negative peaks (red-yellow) correspond to CH pairs with odd numbers of $^{1}J_{CC}$ partners (e.g. CαHα except glycine) and positive peaks (blue) correspond to CH pairs with zero or two $^{1}J_{CC}$ partners. The high intensity positive peak at 3.7 ppm in ^{1}H arises from 10 mM Tris-HCl buffer. Artifacts : The horizontal array of low intensity positive peaks around the Tris peak are truncation ripples from Fourier transformation. The vertical array of low intensity negative peaks at 4.8 ppm arise from residual water after solvent suppression.

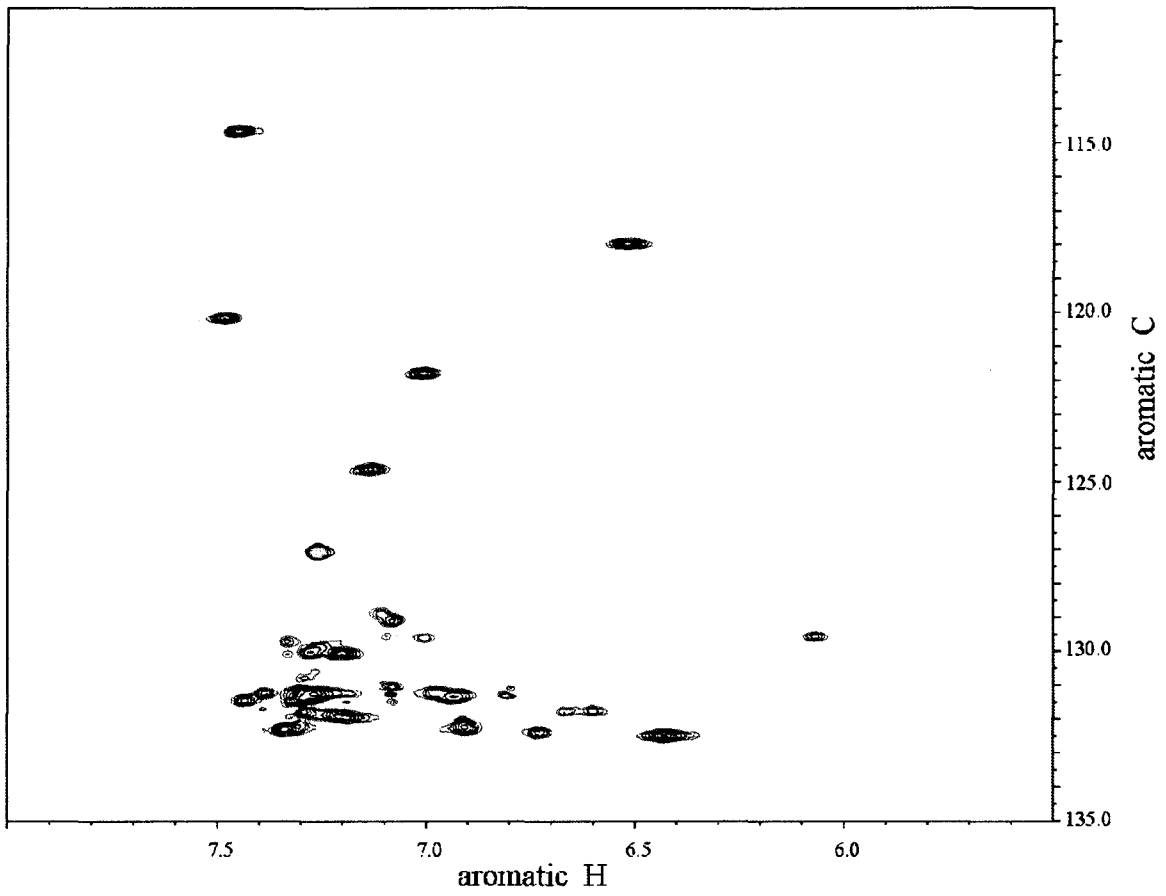


Figure 4.5 2D aromatic ^{13}C HSQC of apoN-androcam

Aromatic region of 1/J ^{13}C-HSQC shows CH pairs with an odd number of $^{1}J_{CC}$ partners as negative peaks (red-yellow) and those with an even number as positive (blue) peaks. $C\delta_1H\delta_1$ of W143 is bonded to only one other carbon (and to the indole nitrogen) and so appears as the only negative peak. Low intensity negative peaks are Fourier transform ripples.

4.2.2 Side chain chemical shifts

I was able to assign a majority of the Cα/Hα correlations for both samples from backbone walk and 2D ^{13}C HSQC experiments. I assigned side chain proton and carbon chemical shifts using the HCCH-COSY (Kay et al., 1990), HCCH-TOCSY (Bax et al., 1990; Kay et al., 1993) and CCH-TOCSY (Bax et al., 1990)experiments that employ a through C-C bond magnetization transfer. In the HCCH-COSY spectrum, at the ^{13}C plane of a given CH pair, the directly attached proton is correlated to protons that are bonded to carbons one C-C bond away. In the TOCSY spectrum, cross peaks are also observed with protons two or three C-C bonds away. In the CCH-TOCSY, correlations are seen to the ^{13}C chemical shifts of the remote carbons, rather than their protons. Starting with methyl peaks in the 2D ^{13}C HSQC spectrum and tracing my way to C_α using the three experiments above, I was able to completely assign methyl and other aliphatic peaks for alanines, valines, leucines, isoleucines and threonines. Because these 3D spectra contain two sets of correlations (A → B and B → A), I was able to confirm these assignments by starting at the previously assigned C_α resonances and tracing out along the sidechains to the methyls. The lack of one-bond ^{13}C neighbors for methionine methyls means that they appear only weakly, if at all, in HCCH-COSY spectra, so I used the 3D LRCC (Bax et al., 1994a) (long range carbon-carbon coupling) experiment to identify the methyl peaks of all nine methionines for apoN androcam and all but M1 for holo androcam.

Although the methyls were well dispersed, side chain assignments for aliphatic parts of residues without methyls were in many cases complicated by chemical shift redundancy. The presence of 12 aspartic acid and 8 asparagine residues, as well as 23 glutamate and 7 glutamine residues results in a very crowded region of Hβ/Cβ and Hγ/Cγ

correlations. Although many of these resonances are unresolved in the 2D spectra, I was able to assign chemical shifts for all aliphatic protons in both samples.

4.2.3 Chemical shift assignments of the aromatic rings

I used two 2D experiments, HBCBCGCDHD (Yamazaki et al., 1993) and HBCBCGCDHDHE (Yamazaki et al., 1993), along with an aromatic ^{13}C HSQC (Figure 3.5) to assign ring protons of phenylalanine, tryptophan and tyrosine residues. These specialized 2D experiments effect magnetization transfers from the aliphatic H_β/C_β pair through the quaternary C_γ to aromatic carbons and finally hydrogens, and they correlate the C_β shift with the H_δ or H_δ/H_ε shifts. I was able to completely assign every ring resonance for W143 and Y138 and every $C_\delta H_\delta$ and $C_\varepsilon H_\varepsilon$ for all Phenylalanines in both the holo and apoN samples. The 2D aromatic HSQC for holo sample did not have enough resolution to assign all Phe $C_\zeta H_\zeta$ pairs, but I was able to assign well-dispersed H_ζ and I assigned a value of $H_\zeta = 7.1$ ppm for phenylalanines 12, 16, 19, 68, 107, 130 and 141. For the apoN sample, only F68 $C_\zeta H_\zeta$ remained unassigned.

The complete chemical shift assignments for holo and apoN samples are presented in Appendix B and C respectively.

4.3 Chemical shift changes between apoN and holo androcam

I shall refer to the high calcium (10mM $CaCl_2$) NMR sample as "holo" and the low salt (17.5 μM $CaCl_2$) sample as "apoN". Comparing the two sets of assignments reveals that overall chemical shift changes are quite small, suggesting minimal changes in the electronic environment experienced by the majority of the protein residues. The difference in resonance chemical shifts $\Delta\delta$ (apoN minus holo) for backbone C_α, C_β, C', H_α, H^N and N are small enough throughout the C-terminal lobe to infer that no conformational change or ligand binding occurs to alter the protein structure; slight shift changes at the very C-terminus likely indicate a modest salt effect. The strongest chemical shift changes in the N-terminal lobe are seen at residues L48-Q62, and we infer these changes to correspond to a localized conformational change mediating a weak Ca^{+2} binding event (Section 3.3). Chemical shift differences in the central linker probably correspond to non-specific effects of Ca^{+2}, since this region contains many acidic residues and is dynamic in solution.

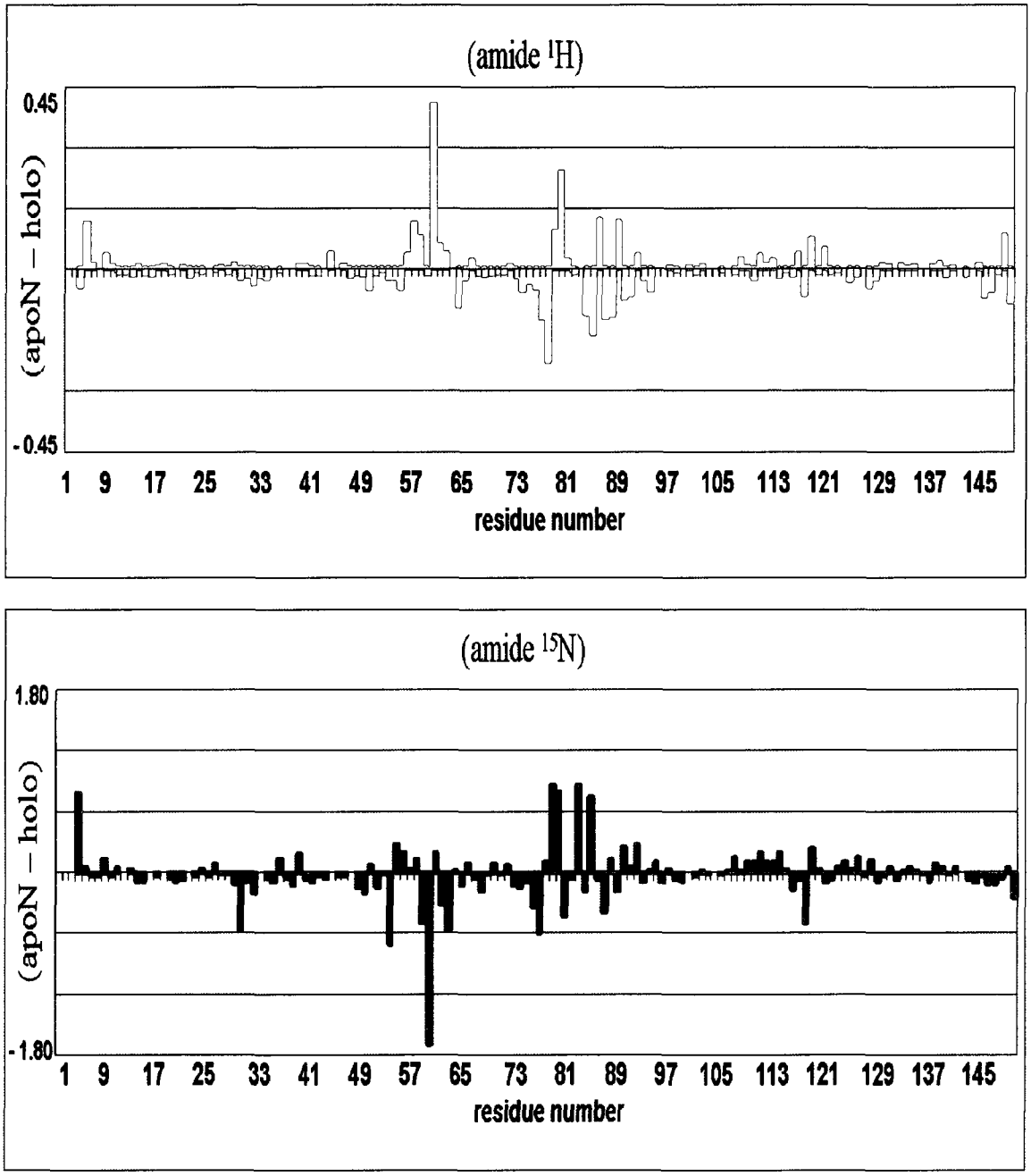


Figure 4.6.A Change in backbone amide proton and nitrogen chemical shifts between apoN-androcam and holo-androcam.

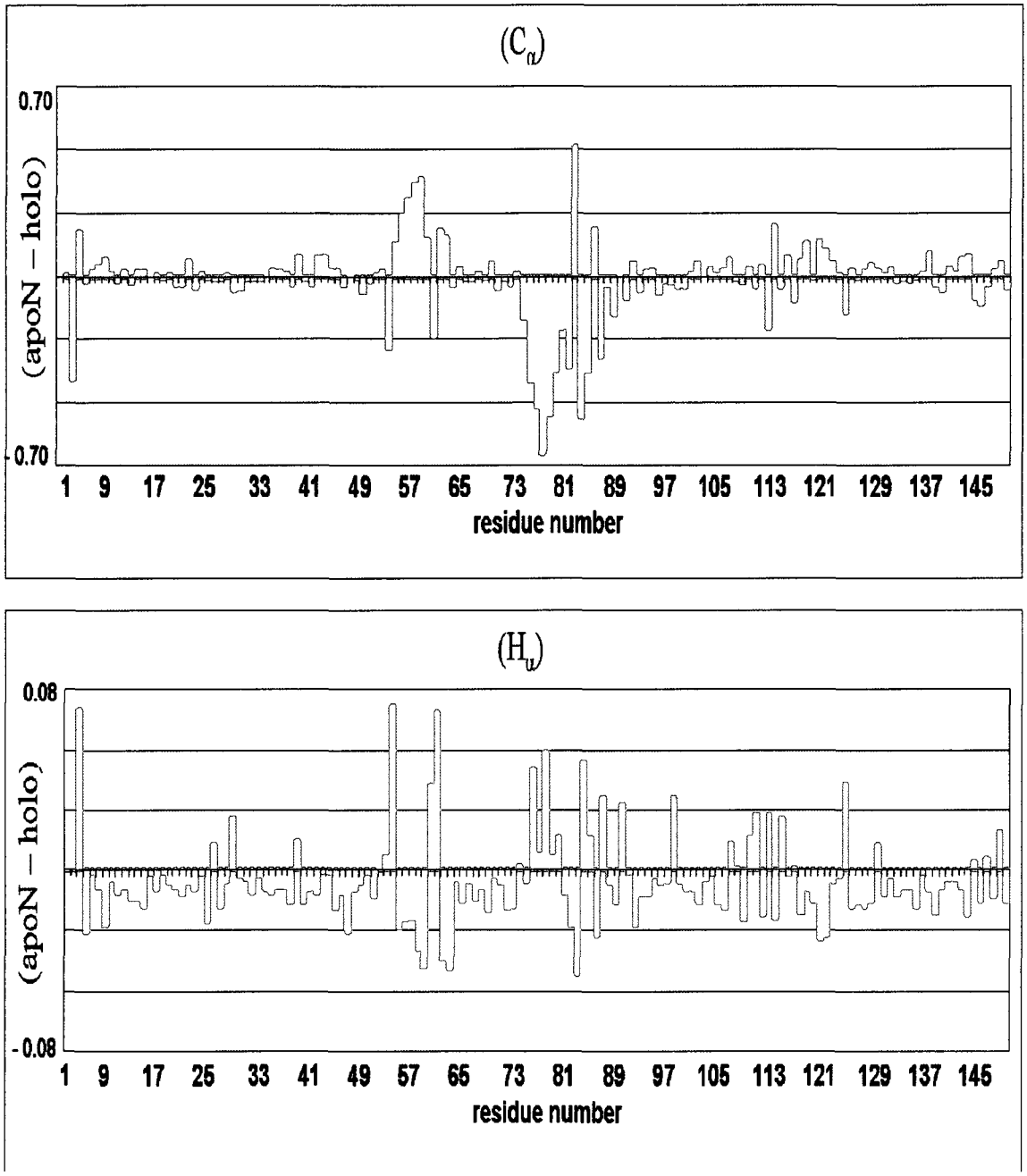


Figure 4.6.B Change in backbone Cα and Hα chemical shifts between apoN-androcam and holo-androcam.

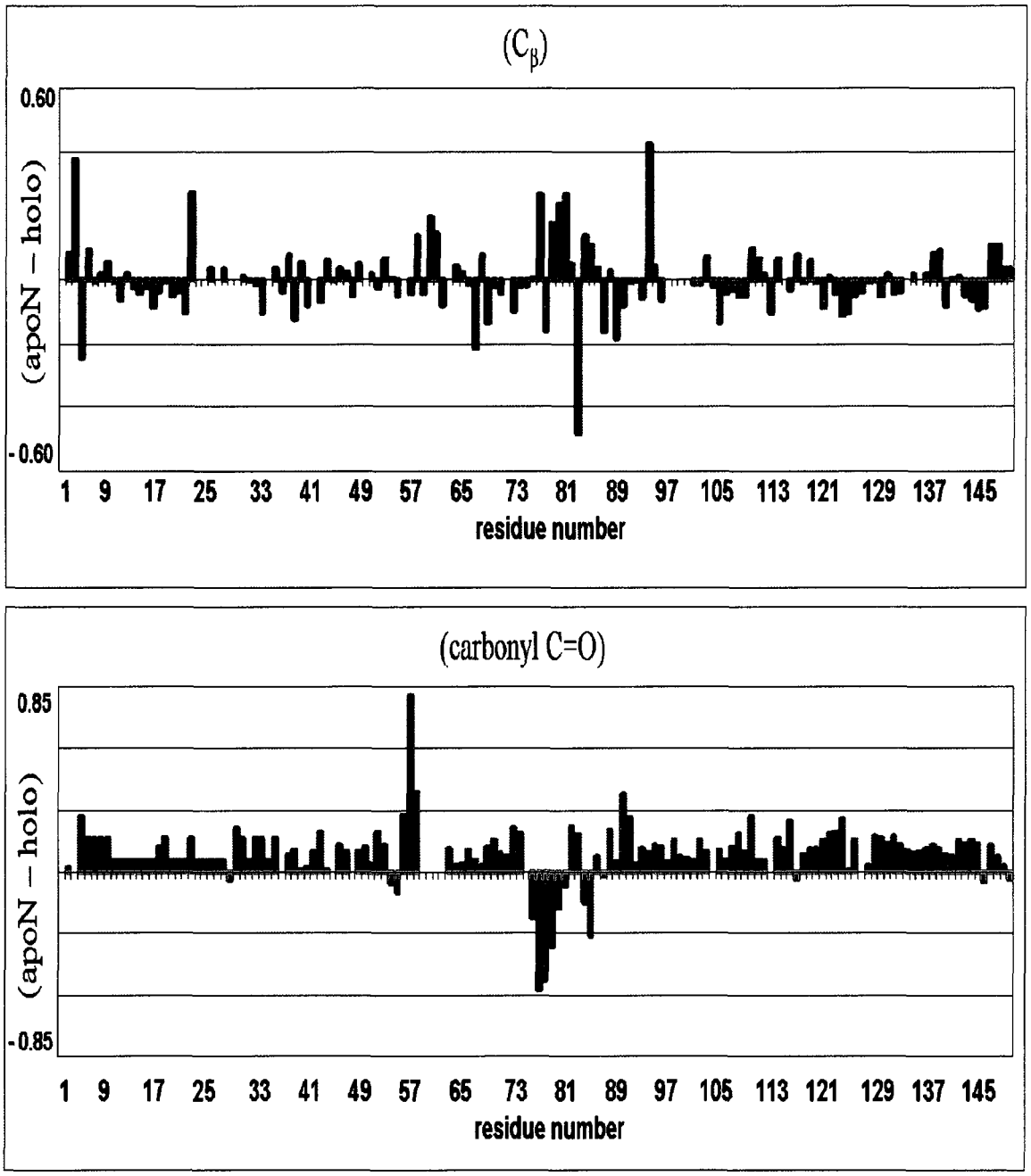


Figure 4.6.C Change in backbone carbonyl carbon and Cβ chemical shifts between apoN-androcam and holo-androcam

4.4 Chemical Shift Index (CSI) analysis

Using a database of about 70 different protein structures, Wishart and colleagues identified correlations between the chemical shifts of Cα, Cβ, Hα and C' resonances and the secondary structure in which the residues are found (Wishart et al., 1991) (Wishart et al., 1992). H_α protons experience an upfield shift (0.15-0.60 ppm) from random coil value when in a helical conformation and a downfield shift when in a beta strand. On the other hand, C_α and C' resonances experience a downfield shift in helices and an upfield shift in beta strands. CSI is a C language based computer program that reads user provided chemical shift information and determines the extent of helicity, beta sheet and random coil structure in the protein (Wishart and Sykes, 1994). I ran the program with chemical shifts of holo and apoN androcam; the regions corresponding to all three types of secondary structures are very similar under holo and apoN conditions. Four α-helices are identified in each lobe, and loop regions of EF hands exhibit short stretches of β-strand and long stretches of random coil. The central linker is non-helical and exhibits flexibility. CSI output files for holo and apoN androcam are shown in Appendix B and C respectively.

4.5 Nuclear Overhauser Effect (NOE) data

I acquired NOE data as 3D NOESY spectra that show ^{1}H-^{1}H dipolar spin relaxation events mediated through space. The intensity of an NOE peak is inversely proportional to the sixth power of the distance separating the two protons: the more intense an NOE peak, the closer the two protons are in space, and protons separated by more than 6 Å or more will not show correlations in a NOESY spectrum. I acquired three types of 3D NOESY spectra that differ in the identity of the hydrogen/heteronucleus pair on which the NOESY spectrum reads out: an aliphatic ^{13}C-separated NOESY, an amide ^{15}N-separated NOESY, and an aromatic ^{13}C-separated NOESY (this last spectrum was acquired for apoN androcam only). Figure 3.7 shows ^{1}H NOE cross peaks from the directly bonded ^{1}H in selected aliphatic, methyl and aromatic carbons and amide nitrogen planes.

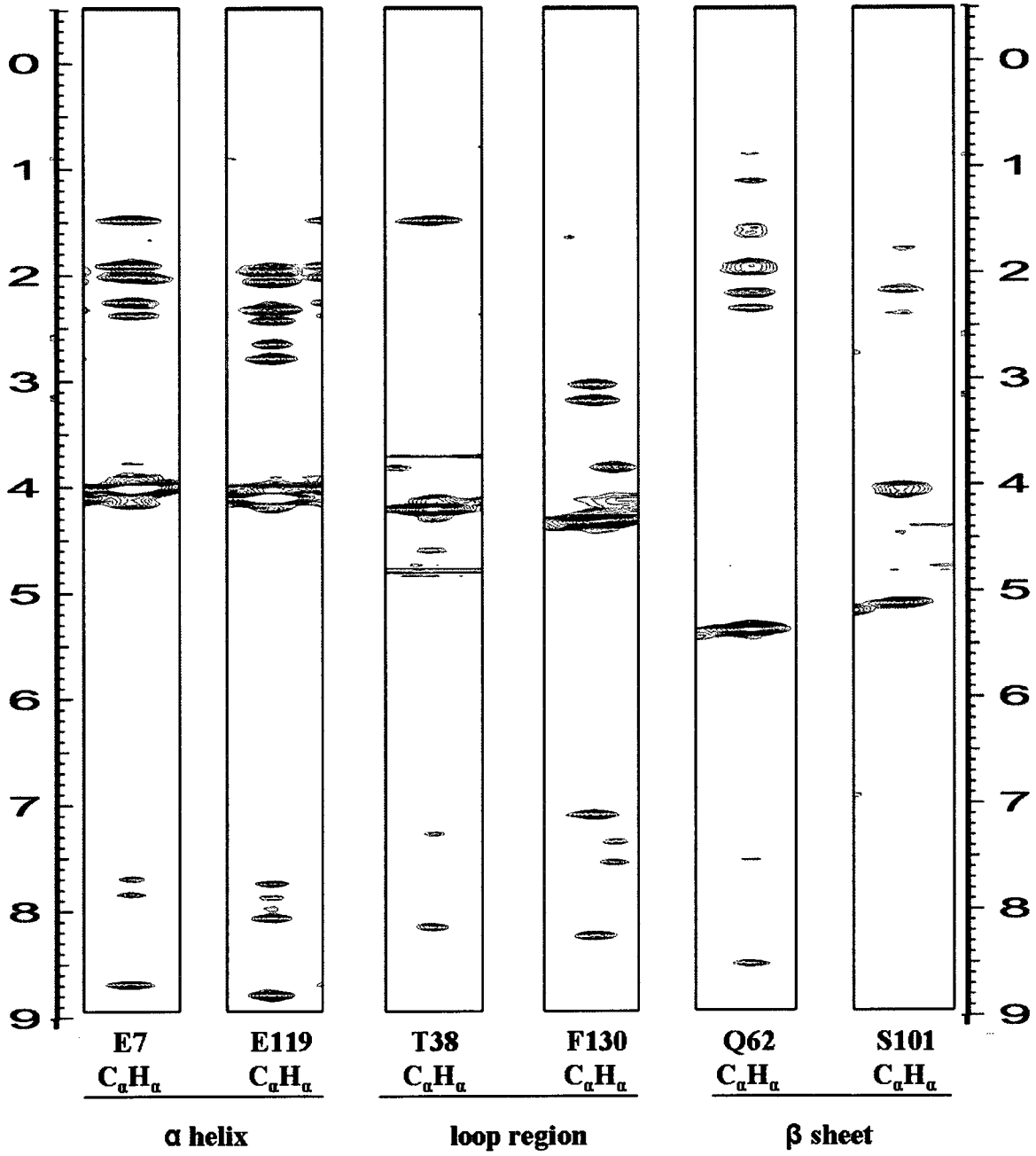


Figure 4.7.A $C_\alpha H_\alpha$ NOE slices from the 3D ^{13}C NOESY spectrum of apoN androcam

Slices from the C_α planes of the residues shown displaying the NOE cross peaks to H_α column. One residue is selected in each of α helix, random coil or beta sheet from N and C lobes.

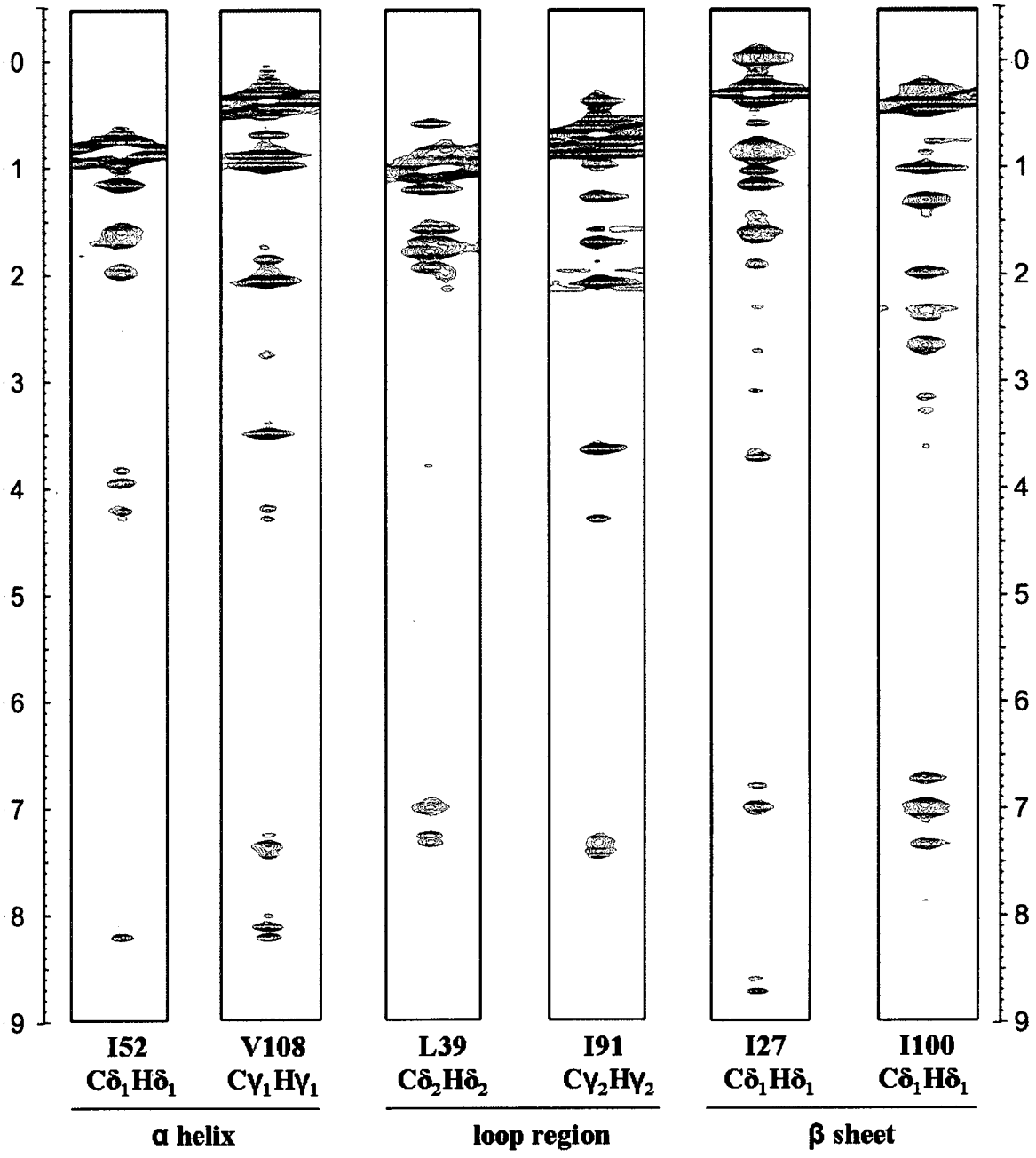


Figure 4.7.B NOE slices from the methyl region of 3D ^{13}C NOESY spectrum of apoN androcam

Slices from the methyl carbon planes of the residues shown displaying the NOE cross peaks to the methyl ^{1}H column. One residue is selected in each of α helix, random coil or beta sheet from N and C lobes.

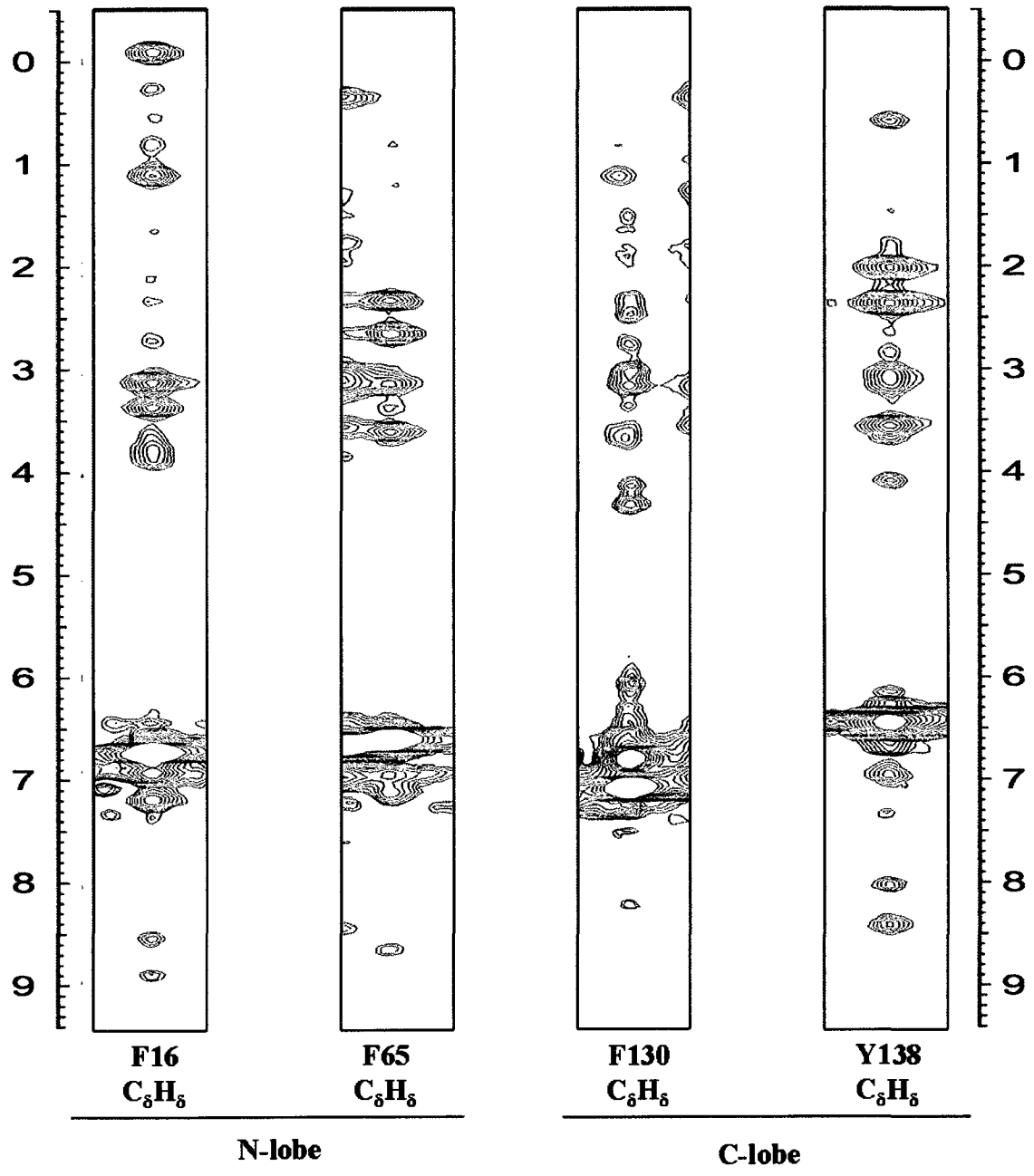


Figure 4.7.C NOE slices for Cδ carbons from the 3D ^{13}C aromatic NOESY spectrum of apoN androcam

Slices from the Cδ planes of the residues shown displaying the NOE cross peaks to Hδ ring proton column. One residue is selected in each of α helix, random coil or beta sheet from N and C lobes.

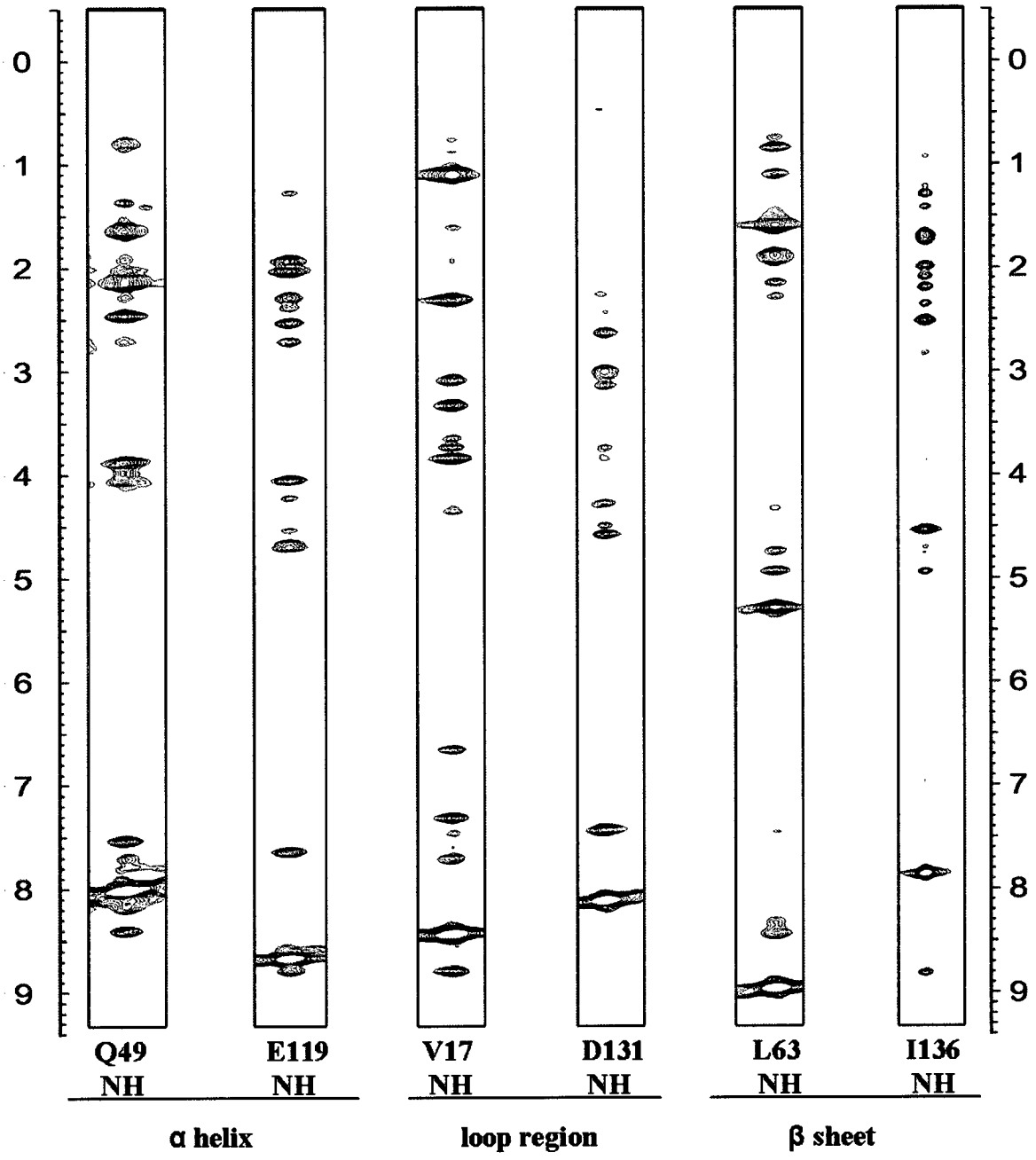


Figure 4.7.D NOE slices of amide N columns from 3D ^{15}N NOESY spectrum of apoN androcam

Slices from the amide N planes of the residues shown displaying NOE cross peaks to the HN proton column. One residue is selected in each of α helix, random coil or beta sheet from N and C lobes.

4.6 $^3J_{HNHA}$ coupling

The 3D HNHA experiment (Vuister and Bax, 1993) measures the 3 bond coupling between the amide ^1H and alpha ^1H. $^3J_{HNHA}$ contains valuable information about the backbone dihedral angle φ as shown by the Karplus relation,

$$J(\phi) = A\cdot\cos^2(\phi-60)+B\cdot\cos(\phi-60)+C$$

where, ϕ is the backbone dihedral angle expressed in degrees and A, B, C are real constants that define the shape of the Karplus curve. Vuister and Bax used the above equation to fit measured values of $^3J_{HNHA}$ for the protein SNase to ϕ angles derived from its crystal structure; the best fit parameterization of the curve gave A=6.51, B=-1.76 and C=1.6 with an rmsd of 0.73 Å. The HNHA experiment has a H_α cross peak and a H^N diagonal peak for every detectable amide, and the J value is calculated from the spectrum using the relation

$$\frac{S_{cross}}{S_{diagonal}} = -\tan^2\left(2\pi\,{}^3J_{HH}\zeta\right)$$

where ζ (typically set to 12.5 ms) is half the time in the pulse sequence for which the H^N-H_α coupling is active, S_{cross} and $S_{diagonal}$ represent integrated peak intensities for the H_α cross peak and HN auto peak respectively. Residues in a helix have a $^3J_{HNHA}$ value of ≤ 4 Hz and for beta sheet and loop regions the value can be as high as 8-10 Hz.

The HNHA data for the holo sample does not have a high enough signal/noise ratio to accurately integrate peaks and its $^3J_{HNHA}$ values and not used in structure calculations. For the apoN sample, I was able to determine the $^3J_{HNHA}$ coupling for all residues except M1, N58, N59 and G61 (which did not exhibit Hα cross peaks due to rapid relaxation) and six residues that have overlapping HN peaks (D14/E31; K26/E47; D131/E145) which make

it difficult to quantify $S_{diagonal}$. I chose not to use $^3J_{HNHA}$ restraints for G23, G70, or G96 because each of these glycines shows a single chemical shift for its two H_α, which makes it impossible to quantify S_{cross} and suggests conformational averaging. The calculated J values for the apoN sample are reported in Appendix C.7; for the holo sample, the J couplings were treated only qualitatively because the spectrum was of moderate quality.

4.7 $^3J_{HNHB}$ coupling

The 3D HNHB experiment (Archer et al., 1991) (Bax et al., 1994b) measures the three bond J coupling between beta protons and the backbone amide nitrogen and provides useful information about the χ_1 torsion angle. To quantitatively use the $^3J_{HNHB}$ information, a reference 2D spectrum needs to be acquired. This is done to ensure that the integrated intensity of an H-N peak in the 2D spectrum and that of an H-N-H_β peak in the 3D spectrum depend on the same way on ^1H and ^{15}N transverse relaxation rates. The $^3J_{HNHB}$ for a given residue is calculated as,

$$\frac{V_{HNB}}{V_{HN}} = \sin^2\left(\pi\Delta\, {}^3J_{HNHB}\right)$$

where, V_{HNB} and V_{HN} are normalized volumes from 3D & 2D spectra calculated as,

$$V_{HNB} = \frac{V_{3D}}{\left(\frac{N_1 \cdot N_2 \cdot N_3}{8}\right)} \qquad V_{HN} = \frac{V_{2D}}{\left(\frac{N_1 \cdot N_2}{4}\right)}$$

where, V_{3D} and V_{2D} are peak volume integrals from the processed spectra and N_1, N_2, and N_3 are the number of real data points in each spectral dimension after Fourier transformation. The delay time Δ is set to an odd multiple of $1/2J_{NH}$ where J_{NH} is the one

bond H-N coupling (~92 Hz) in order to dephase magnetization due to long range J_{HNB} couplings while canceling the effects of evolution under the one bond $^1J_{H-N}$.

For a given beta proton, a $^3J_{HNHB}$ value of >2.5-4.0 Hz indicates *trans* orientation to the amide N and a $^3J_{HNHB}$ value of ~ 1.5 Hz indicates *gauche* orientation. For residues with two distinct H_β peaks that are both in the gauche range, I infer that the $C\gamma$ is *trans* with respect to the amide N; when the two H_β peaks are unresolved but together still correspond to a strongly gauche orientation (<1 Hz), I made the same inference. Usually, in cases where pairs of H_β peaks are unresolved, the calculated $^3J_{HNHB}$ values are intermediate and likely indicate conformational averaging, so no restraint is inferred from these data.

The 3D HN(CO)HB experiment (Grzesiek et al., 1992) (Bax et al., 1994b) provides an analogous three bond correlation of H_β protons to the backbone carbonyl carbon, but the spectrum is less sensitive than the 3D HNHB. For the holo sample, χ_1 restraints were determined by qualitative comparisons of relative peak intensities in the HN(CO)HB and using these findings to complement results from the 3D HNHB spectrum. For the apoN sample, the final inferred χ_1 restraint for a given residue was determined by comparing quantitative $^3J_{HNHB}$ couplings from the 3D HNHB spectrum and the 3D HN(CO)HB spectrum, as well as incorporating other measures of χ_1 such as $^3J_{CN}$ and $^3J_{CCO}$ (see section 4.9). The side chain dihedral χ_1 angle restraints derived from $^3J_{HNHB}$ couplings are presented in appendix C.7 for the apoN sample. Using the HNHB and HN(CO)HB spectra together, I stereospecifically assigned H_β protons in both the holo and apoN androcam samples, as listed in Appendices B.4 and C.4 respectively.

4.8 Long range $^3J_{CC}$ coupling from LRCC data

The same 3D LRCC experiment used to identify the methionine methyls can provide semi-quantitative long range C-C couplings ($^3J_{CC}$) from methyls to other aliphatic carbons. I used the LRCC spectrum of apoN androcam to determine the $^3J_{CC}$ between $C\delta_1$ or $C\delta_2$ and $C\alpha$ for leucine residues and between $C\delta_1$ and either $C\gamma_2$ or $C\alpha$ for isoleucine residues, and from these values I derived χ_2 torsion angle restraints. The ratio of the integrated peak volumes of the correlations to the long range coupled aliphatic C to the CH_3 is given as,

$$\frac{S_{cross}}{S_{auto}} = -\tan^2(2\pi J_{CC}T)$$

where S_{cross} and S_{auto} represent integrated peak volumes of the long range aliphatic C cross peak and the auto methyl peak respectively. During the delay time 2T, dephasing caused by $^1J_{CC}$ and $^nJ_{CC}$ takes place. The time delay T is typically set to $2/^1J_{CC}$ or 57.2 msec to allow the $^1J_{CC}$ to evolve back in-phase leaving only terms that depend on long-range couplings. The calculated values for $^3J_{CC}$ values are reported in Table 4.1 and 4.2. A $^3J_{CC}$ of ~ 3.0 Hz indicates *trans* and ~1.5 Hz indicates *gauche* orientation of the methyl with respect to the long range aliphatic carbon. I was able to determine the χ_2 orientation for $C\delta_1$ methyls of Ile 9, 27, 52, 71, 91, 100, and 125; I was also able to determine how the $C\delta_1$ and $C\delta_2$ methyls of Leu 32, 35, 39, 51, 63, 105, and 112 are oriented relative to their $C\alpha$. The leucine methyls $C\delta_1$ and $C\delta_2$ are allowed to float during calculation and their final stereospecific assignment is determined by ARIA. The χ_2 restraints derived from these data are reported in appendix C.3.

Table 4.1 $^3J_{CC}$ **couplings for isoleucine** δ_1 **methyls**

Residue	Peak volume Cα or $C\gamma_2$	Peak volume $C\delta_1$	$^3J_{CC}$ (Hz)	χ_2 relation
I 9 Cα	-2.490E+08	6.200E+08	3.10	*trans*
I 9 $C\gamma_2$	-3.170E+07	6.200E+08	1.22	*gauche*
I 27 Cα	-1.290E+08	3.380E+08	3.04	*trans*
I 27 $C\gamma_2$	-3.060E+07	3.380E+08	1.60	-
I 52 Cα	-1.690E+08	3.260E+08	3.42	*trans*
I 52 $C\gamma_2$	-4.390E+07	3.260E+08	1.93	-
I 71 Cα	-9.730E+07	1.830E+08	3.46	*trans*
I 71 $C\gamma_2$	-	1.830E+08	0.00	*gauche*
I 91 Cα	-1.160E+08	2.980E+08	3.06	*trans*
I 91 $C\gamma_2$	-3.990E+07	2.980E+08	1.92	-
I 100 Cα	-5.930E+07	1.170E+08	3.39	*trans*
I 100 $C\gamma_2$	-1.000E-02	1.170E+08	0.00	*gauche*
I 110 Cα	-6.060E+08	1.960E+09	2.78	avg
I 110 $C\gamma_2$	-1.940E+08	1.960E+09	1.67	avg
I 121 Cα	-6.520E+07	3.410E+08	2.26	avg
I 121 $C\gamma_2$	-4.120E+07	3.410E+08	1.84	avg
I 125 Cα	-1.180E+08	3.190E+08	3.00	*trans*
I 125 $C\gamma_2$	-3.240E+07	3.190E+08	1.69	-
I 136 Cα	-4.950E+07	2.190E+08	2.44	avg
I 136 $C\gamma_2$	-2.410E+07	2.190E+08	1.76	avg
I 145 Cα	-1.050E+08	3.980E+08	2.60	avg
I 145 $C\gamma_2$	-5.190E+07	3.980E+08	1.90	avg

Table 4.2 $^3J_{CC}$ **couplings for leucine** δ_1 **and** δ_2 **methyls**

Residue	**Peak volume Cα**	**Peak volume $C\delta_1$ or $C\delta_2$**	**$^3J_{CC}$ (Hz)**	**χ_2 relation**
L4 $C\delta_1$	-5.840E+07	1.050E+09	1.27	avg
L4 $C\delta_2$	3.160E+07	-9.290E+07	2.90	avg
L32 $C\delta_1$	-6.530E+06	1.690E+08	1.07	*gauche*
L32 $C\delta_2$	5.470E+07	-7.790E+07	3.83	*trans*
L35 $C\delta_1$	-9.600E+06	1.330E+08	1.44	*gauche*
L35 $C\delta_2$	2.840E+07	-2.960E+07	4.25	*trans*
L39 $C\delta_1$	-7.140E+06	1.690E+08	1.11	*gauche*
L39 $C\delta_2$	2.710E+07	-3.180E+07	4.09	*trans*
L48 $C\delta_1$	-1.270E+08	5.620E+08	2.44	avg
L48 $C\delta_2$	-1.460E+08	5.970E+08	2.52	avg
L51 $C\delta_1$	-2.920E+07	3.810E+08	1.48	*gauche*
L51 $C\delta_2$	2.360E+08	-4.210E+08	3.53	*trans*
L63 $C\delta_1$	4.260E+07	-4.750E+08	1.60	*gauche*
L63 $C\delta_2$	1.010E+08	-2.740E+08	2.99	*trans*
L105 $C\delta_1$	-2.430E+07	2.840E+08	1.56	*gauche*
L105 $C\delta_2$	1.250E+08	-1.960E+08	3.70	*trans*
L112 $C\delta_1$	-4.200E+07	4.510E+08	1.63	*gauche*
L112 $C\delta_2$	1.620E+08	-3.100E+08	3.43	*trans*

4.9 $^3J_{CCO}$ and $^3J_{CN}$ couplings

$^3J_{CCO}$ and $^3J_{CN}$ between side chain γ methyls ^{13}Cs and backbone carbonyl carbons or amide nitrogens can be measured using spin-echo difference experiments (Bax et al., 1994b) that encode the magnitude of the three bond J coupling between backbone C=O and backbone amide N to methyls carbons in the intensity differences of 2D spectra acquired with the couplings refocused or completely active. I used spin-echo difference $^3J_{CN}$ and $^3J_{CCO}$ data for the apoN sample to determine the stereospecific assignments of valine CH_3 resonances as well as the χ_1 torsion angle in valine, threonine and isoleucine residues. The pulse scheme is similar to the CT ^{13}C HSQC experiment (ref) with the constant time evolution period 2T set to $2/^1J_{CC}$ (57.2 ms) to suppress the effect of one bond C-C couplings (~35 Hz). Two spectra are acquired for each experiment. In the first spectrum, the $^3J_{CCO}$ or $^3J_{CN}$ is refocused by suitably positioning a 180° refocusing pulse in the constant time evolution period. In the second spectrum, the 180° refocusing pulse is repositioned to allow the coupling to be <u>active</u> throughout the period 2T. Couplings are given by the ratio of the difference of the intensities of peaks in the two spectra and the intensities in the refocused spectrum, as

$$\left(\frac{S_{refocused} - S_{active}}{S_{refocused}}\right) = 2\sin^2(\pi J_{CX} T)$$

where $S_{refocused} \equiv$ integrated peak intensity in the 1st spectrum ($^3J_{CX}$ refocused)

$S_{active} \equiv$ integrated peak intensity in the 2nd spectrum ($^3J_{CX}$ active)

$T \equiv$ half of the constant time evolution period ($1/^1J_{CC}$ = 28.6 ms)

$^3J_{CCO}$ and $^3J_{CN}$ values are reported in Tables 4.3. A $^3J_{CN}$ of ~ 2.0 Hz implies "trans" whereas < 0.7 Hz implies "gauche" orientation relative to the amide N. A $^3J_{CCO}$ of ~ 4.0

Hz implies "trans" and < 1.0 Hz implies "gauche" orientation relative to the carbonyl carbon. Table 4.4 shows stereospecific orientation of the methyls with $^3J_{HNHB}$ couplings. The χ_1 restraints are reported in appendix C. From Table 4.4 I was able to stereospecifically assign $C\delta_1$ and $C\delta_2$ methyls of Valine 108 and 142.

Table 4.3 $^3J_{CN}$ and $^3J_{CCO}$ coupling for Ile, Thr and Val methyls

Methyl peak		$^3J_{CN}$ refocused volume	$^3J_{CN}$ active volume	$^3J_{CN}$ (Hz)	$^3J_{CCO}$ refocused volume	$^3J_{CCO}$ active volume	$^3J_{CCO}$ (Hz)
I9	$C\gamma_2$	7.76E+07	5.31E+06	2.04	1.46E+08	2.74E+06	1.06
I27	$C\gamma_2$	8.40E+07	1.14E+06	0.91	1.62E+08	2.52E+07	3.10
I52	$C\gamma_2$	7.81E+07	3.25E+06	1.59	1.42E+08	1.89E+07	2.86
I71	$C\gamma_2$	9.06E+07	6.57E+06	2.10	1.33E+08	-	0.00
I91	$C\gamma_2$	9.07E+07	6.65E+06	2.11	1.72E+08	2.43E+06	0.92
I100	$C\gamma_2$	5.30E+07	4.38E+06	2.25	1.09E+08	-	0.00
I110	$C\gamma_2$	1.85E+08	1.06E+07	1.87	3.54E+08	1.27E+07	1.47
I121	$C\gamma_2$	1.02E+08	6.61E+06	1.99	1.86E+08	2.47E+06	0.90
I125	$C\gamma_2$	8.96E+07	8.31E+06	2.38	1.65E+08	2.99E+06	1.05
I136	$C\gamma_2$	4.65E+07	3.46E+06	2.13	9.31E+07	-	0.00
I145	$C\gamma_2$	1.10E+08	2.43E+06	1.16	1.97E+08	1.36E+07	2.05
T24	$C\gamma_2$	1.13E+08	-	0.00	1.29E+08	1.25E+07	2.44
T29	$C\gamma_2$	6.50E+07	-	0.00	1.24E+08	2.03E+07	3.18
T34	$C\gamma_2$	9.60E+07	3.04E+06	1.38	1.61E+08	-	0.00
T38	$C\gamma_2$	1.15E+08	2.76E+06	1.20	2.05E+08	-	0.00
T44	$C\gamma_2$	1.03E+08	9.94E+05	0.76	1.13E+08	1.75E+07	3.09
T66	$C\gamma_2$	2.88E+08	6.05E+06	1.13	4.16E+08	4.50E+06	0.81
T79	$C\gamma_2$	3.83E+08	9.23E+06	1.21	8.92E+08	4.92E+07	1.83
T81	$C\gamma_2$	2.35E+08	1.74E+06	0.67	4.53E+08	1.72E+07	1.52
T117	$C\gamma_2$	2.61E+08	5.00E+06	1.08	4.22E+08	5.82E+07	2.92
V17	$C\gamma_1$	2.02E+08	4.46E+06	1.16	3.92E+08	2.80E+07	2.09
V17	$C\gamma_2$	1.59E+08	1.58E+06	0.77	2.71E+08	1.81E+07	2.02
V108	$C\gamma_1$	7.00E+07	4.29E+06	1.93	1.33E+08	-	0.00
V108	$C\gamma_2$	7.97E+07	-	0.00	1.54E+08	2.54E+07	3.20
V116	$C\gamma_1$	3.25E+08	6.02E+06	1.06	5.88E+08	8.72E+06	0.95
V116	$C\gamma_2$	2.50E+08	2.67E+06	0.80	4.50E+08	3.52E+07	2.18
V142	$C\gamma_1$	8.60E+07	7.39E+05	0.72	1.41E+08	2.92E+07	3.60
V142	$C\gamma_2$	1.41E+08	7.36E+06	1.78	2.58E+08	2.13E+06	0.71

Table 4.4 **$^3J_{CN}$, $^3J_{CCO}$ and $^3J_{HNHB}$ couplings with stereospecific orientation for Ile, Thr and Val methyls.**

Methyl peak		$^3J_{CN}$ (Hz)	$^3J_{CCO}$ (Hz)	$^3J_{HNHB}$ (Hz)	Orientation around χ_1		
					CH_3 to N	CH_3 to C=O	N to HB
I9	$C\gamma_2$	2.04	1.06	1.38	*trans*	*gauche*	*gauche*
I27	$C\gamma_2$	0.91	3.10	3.41	*gauche*	*gauche*	*trans*
I52	$C\gamma_2$	1.59	2.86	n/a	avg	avg	avg
I71	$C\gamma_2$	2.10	0.00	2.6	*trans*	*gauche*	*gauche*
I91	$C\gamma_2$	2.11	0.92	2.51	*trans*	*gauche*	*gauche*
I100	$C\gamma_2$	2.25	0.00	1.84	*trans*	*gauche*	*gauche*
I110	$C\gamma_2$	1.87	1.47	2.81	*trans*	*gauche*	*gauche*
I121	$C\gamma_2$	1.99	0.90	2.48	*trans*	*gauche*	*gauche*
I125	$C\gamma_2$	2.38	1.05	n/a	*trans*	*gauche*	*gauche*
I136	$C\gamma_2$	2.13	0.00	2.16	*trans*	*gauche*	*gauche*
I145	$C\gamma_2$	1.16	2.05	2.93	*gauche*	*gauche*	*trans*
T24	$C\gamma_2$	0.00	2.44	2.93	*gauche*	*gauche*	*trans*
T29	$C\gamma_2$	0.00	3.18	n/a	*gauche*	*trans*	*gauche*
T34	$C\gamma_2$	1.38	0.00	1.48	avg	avg	avg
T38	$C\gamma_2$	1.20	0.00	2.40	avg	avg	avg
T44	$C\gamma_2$	0.76	3.09	2.62	*gauche*	*trans*	*gauche*
T66	$C\gamma_2$	1.13	0.81	1.71	avg	avg	avg
T79	$C\gamma_2$	1.21	1.83	2.84	avg	avg	avg
T81	$C\gamma_2$	0.67	1.52	2.72	*gauche*	*gauche*	*trans*
T117	$C\gamma_2$	1.08	2.92	2.93	*gauche*	*gauche*	*trans*
V17	$C\gamma_1$	1.16	2.09	3.23	avg	avg	avg
V17	$C\gamma_2$	0.77	2.02	3.23	avg	avg	avg
V108	$C\gamma_1$	1.93	0.00	2.15	*trans*	*gauche*	*gauche*
V108	$C\gamma_2$	0.00	3.20	2.15	*gauche*	*trans*	*gauche*
V116	$C\gamma_1$	1.06	0.95	3.23	avg	avg	avg
V116	$C\gamma_2$	0.80	2.18	3.23	avg	avg	avg
V142	$C\gamma_1$	0.72	3.60	2.10	*gauche*	*trans*	*gauche*
V142	$C\gamma_2$	1.78	0.71	2.10	*trans*	*gauche*	*gauche*

4.10 Hydrogen bond restraints

CSI analysis of chemical shift information and $^{3}J_{HNHA}$ couplings provide two experimental measures that can be used to identify α-helical regions or β-strands in proteins. I used these two pieces of information together to establish a consensus secondary structure for the two androcam samples and then wrote hydrogen bond distance restraints between backbone carbonyl oxygens of residue *i* and the amide hydrogens and nitrogens of residue *i*+4, which form hydrogen bonds in α-helices. The hydrogen bonding restraints for the holo and apoN structures are shown in appendix B.2 and C.2 respectively. Similar restraints that improve hydrogen bonding geometry in short antiparallel β-strands were added to the structure calculations only after establishing that the hydrogen bonding partners could be identified on the basis of NOE information.

5. STRUCTURE DETERMINATION

5.1 ARIA

ARIA (Ambiguous Restraints for Iterative Assignments) is a software package that assigns or identifies ambiguous NOE peaks from an input list and simultaneously, in an iterative fashion, determines the structure of the protein being studied (Linge et al., 2001). The conceptual advantages offered by this approach are described at the end of Chapter 4.1. Here I focus upon the implementation of the protocol, which can be done with only minimal special knowledge on the part of the user, and I will also describe the analysis of the output, which is necessarily somewhat complicated.

In a web browser interface, the user enters into appropriate fields of the file "new.html" the paths to individual files that contain NMR data such as NOE peak intensities, J couplings, and residual dipolar couplings (RDCs), as well as files that contain restraints inferred from experimental data such as torsion angle restraints or hydrogen bond distance restraints. The NOE peak files that are listed in "new.html" contain the chemical shifts and integrated intensities of the peaks, and they may contain tentative peak assignments, but this is not required. ARIA will assign these peaks in an iterative fashion using the chemical shift list file: a (complete) list of ^{1}H, ^{15}N, and ^{13}C shifts for the protein. In addition to pointing to this shift list, "new.html" specifies tolerance limits for identifying peaks by their chemical shifts (making NOE assignments): that is, the maximal amount by which the value in the shift list may differ from the shift of an NOE peak and still be considered a potential candidate for assignment. The user can edit these tolerances, and work by others has shown that the ARIA procedure handles overly large tolerances better than overly small ones.

Upon executing the file "new.html", ARIA creates the structure calculation file "run.cns", generates a set of directories to store user input files, load CNS protocols and output calculated structure and analysis files. At this stage, the user edits the file "run.cns" to specify information for the simulated annealing search protocol to be used for structure calculations. Details include numerical constants, a choice of Cartesian or torsion angle molecular dynamics simulation, a choice of NOE calibration methods, the number of structures to calculate, and options for how the final structures should be analyzed and compared. Once this file has been edited to the user's satisfaction, the ARIA assignment and structure determination run can be started. The flow chart for ARIA calculations is shown in Figure 5.1.

A very significant bottleneck in solving structures of biological molecules using NMR is the assignment of NOE peaks. Because NOE peaks arise from through-space interactions, any hydrogen in the molecule could interact with any other hydrogen, depending on the fold. With hundreds or thousands of hydrogens, chemical shift redundancies in proteins make it difficult to determine which protons give rise to a particular NOE cross peak, and mis-assignment of an NOE peak would undoubtedly bias the calculation and produce incorrect structures. Moreover, a single NOE peak might possibly arise from more than one through-space proton-proton interaction that happen to overlap in chemical shift; failing to account for this possibility could result in over-estimating the magnitude of one NOE and not accounting for others at all. Because my structure calculations involve several thousand NOE peaks, curating this list for possible assignment errors would be a gargantuan task. Instead, we start from fully ambiguous 'assignments': we assume that any hydrogen within the tolerance bounds of an NOE peak

may contribute to the observed NOE intensity. ARIA then uses an iterative bootstrapping technique to reduce ambiguities in these NOE assignments using structural information from preliminary CNS structure calculations. The preliminary structures are based on Ambiguous Distance Restraints (ADRs) (Nilges, 1993; Nilges, 1995): rather than ignoring NOEs whose identities are ambiguous, the calculation protocol includes all possible peak identities simultaneously in calculating the corresponding energy gradients. The simulated annealing protocol yields 20 structures that satisfy these ambiguous restraints and ARIA scripts then examine these structures to determine which pairs of atoms in each ambiguous restraint are close enough to contribute to the observed NOE peak. Atoms on the ambiguous restraint list that are never close in space (across 20 structures) are deleted from the list, reducing the assignment ambiguity. Each successive round of structure calculations contains less total ambiguity in the NOE ADRs, resulting in families of structures that agree with one another more closely. Because ARIA exhaustively checks all possible NOE assignments after each round of structure calculation, not just all assignments left from the previous round, potential assignments that are mistakenly eliminated in early rounds are automatically re-introduced if other data cause the atoms in question to come within NOE distance of one another in subsequent calculations. ARIA thus provides a statistically robust and fully automated process for assignment of NOEs, completely eliminating the need for the user to assign any NOEs. ARIA does identify and flag data that are inconsistent with the final assignments and structures, and the user must curate this (very small) set of peaks to determine if the peak has been mis-picked, mis-integrated, or does not corresponds to a protein peak (for instance, the DSS standard).

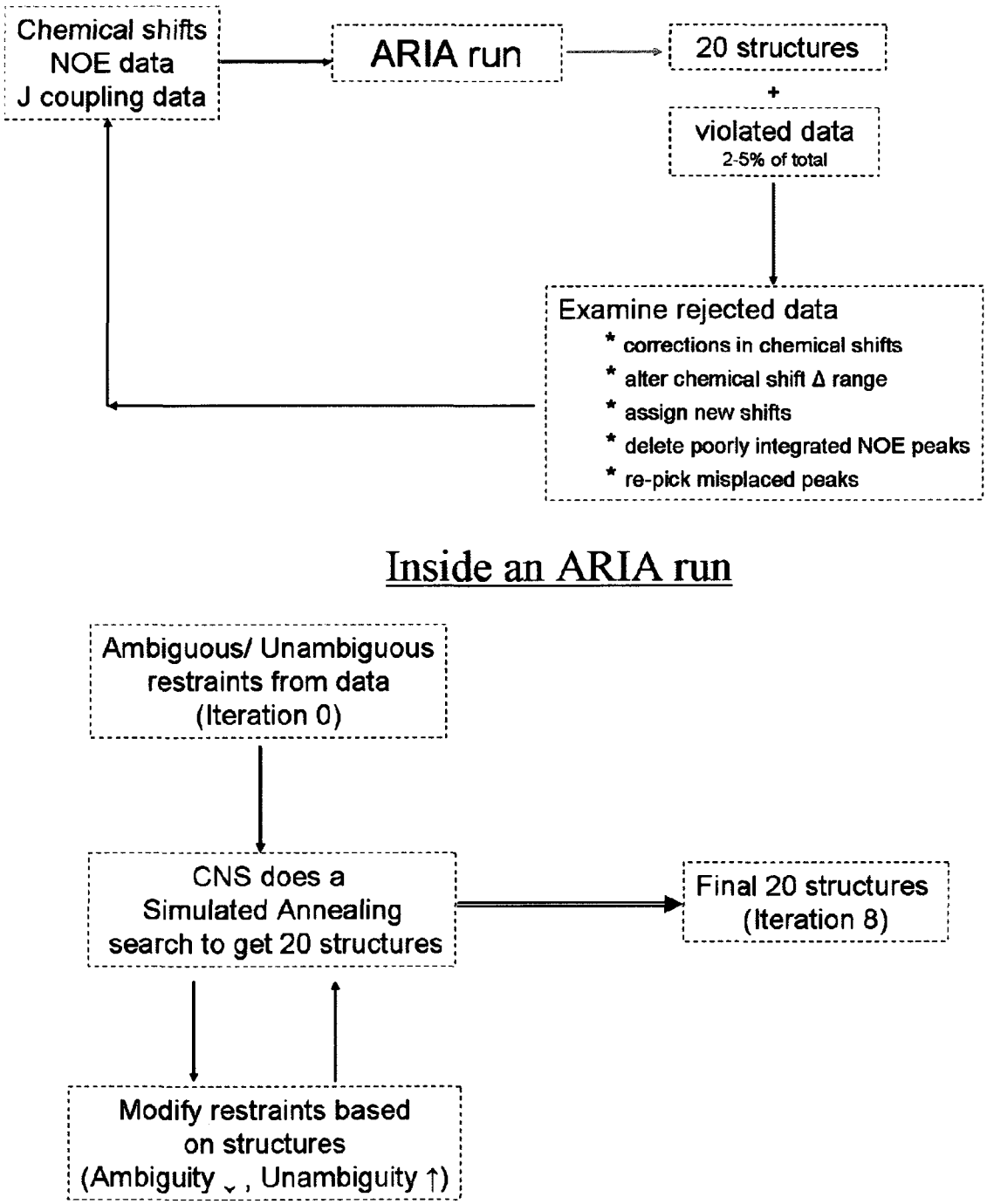


Figure 5.1 Flowchart representation of the working of the program ARIA

The user provides input data in the form of NOE peaks, J couplings, and chemical shifts for all hydrogens (and available heteroatoms). ARIA iteratively performs a simulated annealing search in CNS, assigning restraints at each iteration, to generate a final set of 20 structures. By analyzing the ARIA output that has the list of restraints violated and unused data, the user can identify erroneous shift assignments, re-integrate NOE peaks or alter calculation parameters to arrive at a final set of structures most consistent with the data.

In practice, ARIA starts with a small initial set of unambiguous NOE assignments for peaks that have uniquely identifiable chemical shifts and a large set of ambiguous assignments. Using the unambiguous NOEs it calculates a set of energy minimized structures in iteration 'i' and uses the lowest energy structures (typically 7) to further assign more of the ambiguous NOEs that will be used as unambiguous NOEs in iteration 'i+1'. Thus with each succeeding iteration in ARIA, there is less and less ambiguity of NOEs helping to arrive at a set of structures more and more consistent with the data.

I have used ARIA 1.2, which does a total of 8 iterations of a simulated annealing search in CNS followed by refinement in an explicit water shell (Linge and Nilges, 1999). The final analysis includes calculation of a set of average structures and determining any violations of NOE, J coupling, and torsion angle data. The summary file "overview.aria" lists the following:

1. the total number of short, medium and long-range NOEs used in the calculations,
2. the energy contributions in the final structures from the data-based pseudo-potentials (NOEs, J couplings, torsion restraints) and from the idealized geometrical parameters (bond lengths, bond angles, dihedrals, Van der Waals)
3. root mean square deviations from the data and from idealized geometrical parameters
4. the list of rejected NOEs peaks not used in structure calculations

5.2 Structure of androcam

The structures of androcam at high (holo) and low (apoN) [Ca^{+2}] were both calculated as described in Section 5.1 using the program ARIA. Structure calculations in CNS (called by ARIA) perform a simulated annealing search protocol that begins with a high energy linearized protein molecule and does molecular dynamics simulation (MD) steps that search conformational space to arrive at low energy structures that closely satisfy the experimental data. The MD protocol is repeated with different initial velocities, giving a family of structures that we superimpose with respect to one another to see how well they have converged to a single fold. The family of 20 lowest energy structures out of 50 calculated structures is used for comparison.

Like calmodulin, androcam is a bi-lobed protein with distinct N and C terminal domains connected by a flexible central linker that uncouples them structurally. The N-lobe of apoN androcam is defined with very high precision: the Cα atoms of residues 1-74 can be superimposed for 20 structures with an rmsd of 0.29 Å (Figure 5.2). The C-lobe of apoN androcam is even more precisely defined, with rmsd = 0.21 Å for the Cα atoms of residues 85-148 (Figure 5.2). The family of structures agrees very closely in the helical regions and only somewhat less closely in the loop regions. The lobes of holo androcam are slightly better defined than the apoN structures, with the N-lobe rmsd = 0.26 Å and the C-lobe rmsd = 0.15 Å (Figure 5.3). As with apoN, the family of structures agrees very closely in the helical regions and only somewhat less closely in the loop regions. The structure determination statistics for apoN and holo androcam are presented in Table 5.1; with 32 or 37 NOE restraints per residue, two dihedral restraints per residue, essentially no violations, and excellent geometry, these are very high quality structures.

Table 5.1 Structural statistics for holo and apoN androcam structures

	holo	apoN
NMR restraints		
All NOEs	4839	5463
Intra residue NOEs	1736	1947
Sequential NOEs	1131	1120
Medium-range NOEs	804	980
Long-range NOEs	1168	1416
Hydrogen bond restraints	51	51
φ dihedral restraints	103	n.a.
ψ dihedral restraints	103	103
J couplings (HNHA)	n.a.	126
side chain torsion restraints	77	98
rmsd from ideal geometry		
bond length (Å)	0.002 ± 0.000	0.003 ± 0.000
bond angles (deg)	0.385 ± 0.006	0.463 ± 0.008
impropers (deg)	0.286 ± 0.007	0.408 ± 0.013
rmsd from data		
noe (Å)	0.014 ± 0.000	0.021 ± 0.004
cdih (deg)	0.498 ± 0.028	0.870 ± 0.056
J couplings(Hz)	n.a.	0.817 ± 0.042
violations per structure		
NOE	0.0	0.3
cdih	0.0	1.0
coupling	n.a.	26.9

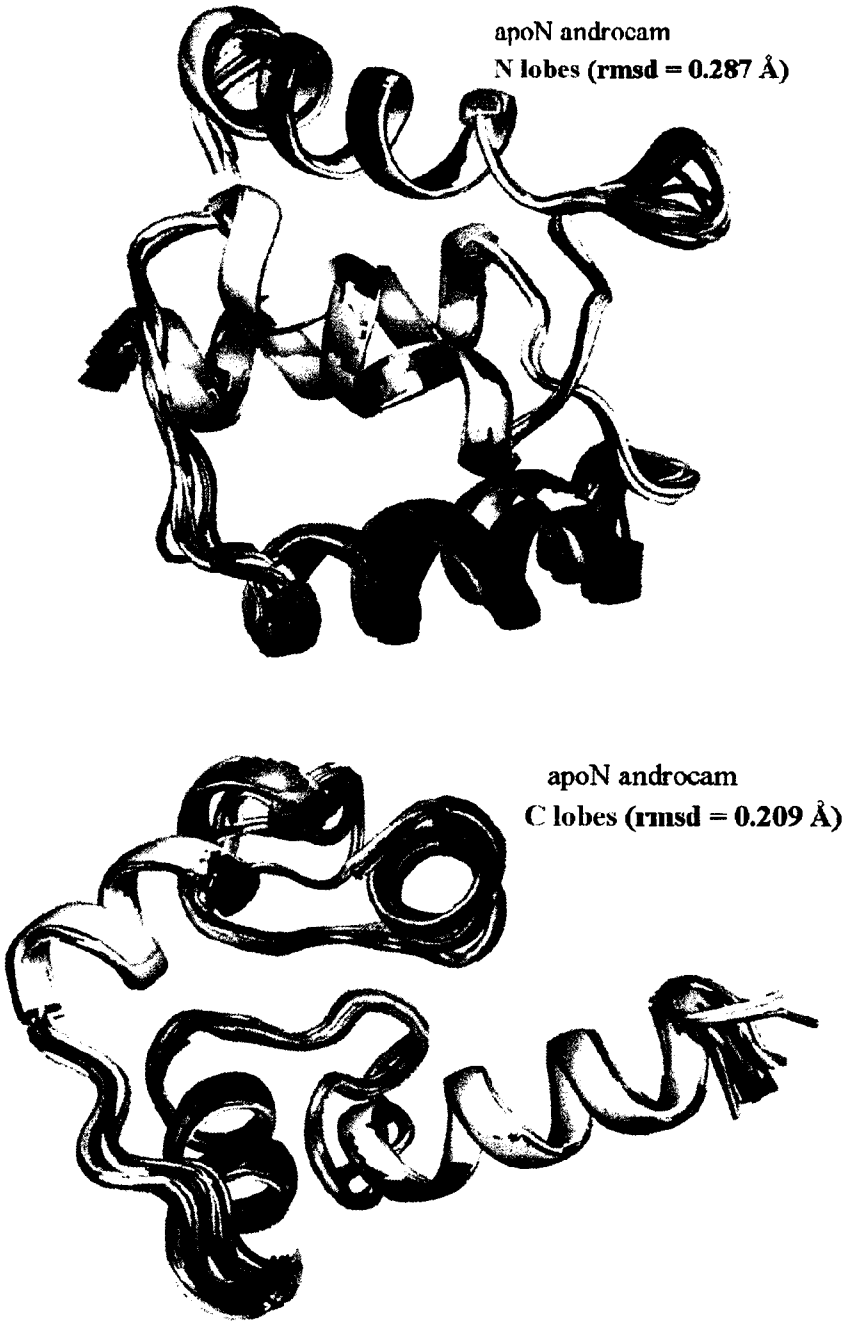


Figure 5.2 Structures of apoN androcam

Independent superpositions of the N and C lobes of apoN androcam for the 20 lowest energy structures out of a total of 50 structures. The CNS protocol 'wellordered.inp' is used for alignment. The lobes are superimposed using the Cα atoms of residues 4-75 for the N lobe and residues 83-148 for the C lobe.

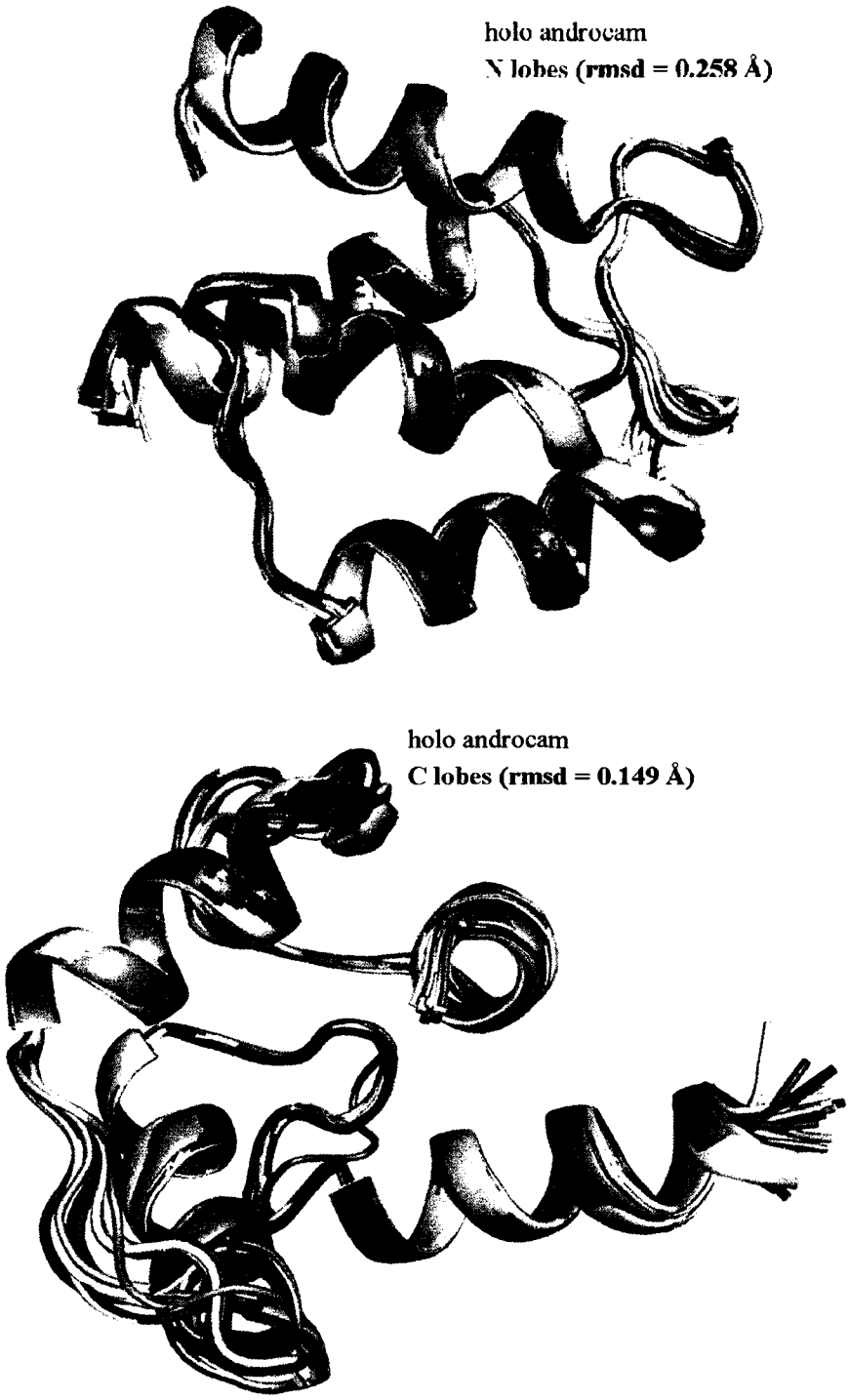


Figure 5.3 Structures of holo androcam

Independent superpositions of the N and C lobes of holo androcam for the 20 lowest energy structures out of a total of 50 structures. The CNS protocol 'wellordered.inp' is used for alignment. The lobes are superimposed using the Cα atoms of residues 4-77 for the N lobe and residues 83-148 for the C lobe.

5.2.1 Androcam fold is maintained over the entire physiological range of [Ca^{+2}]

Because the N and C lobes of holo androcam are so well defined, we can use the average structure of the 20 holo structures and compare it with the family of final 20 structures of apoN androcam. I found that the structure in each lobe of apoN androcam is very similar to that in holo androcam (N-lobe rmsd = 1.68 Å, C-lobe rmsd = 1.29 Å) Figure 5.4. The differences are larger from one family to the other than within each family, but this is likely due to the different restraints used in the calculations. Because we see no evidence for additional Ca^{+2} binding to the C-lobe, we take the C-lobe rmsd of 1.29 Å as indicative of no detectable differences between families. Using this baseline, it seems that the N-lobes do differ very slightly between the low and high calcium state – but this should be contrasted with calmodulin, where the low calcium 'closed' state and the high calcium 'open' state superimpose with an rmsd of ~ 6 Å (not shown). This comparison indicates that the N and C lobes of androcam are constitutively locked in essentially the same configuration at 17.5 μM Ca^{+2} and at 10 mM Ca^{+2}. Calmodulin undergoes a gross conformational switch in each lobe in response to changes in [Ca^{+2}], thereby transducing signals. Androcam on the other hand seems to be completely insensitive to [Ca^{+2}] in the μM to mM range and therefore incapable of responding to calcium signals (at least in the way that calmodulin does).

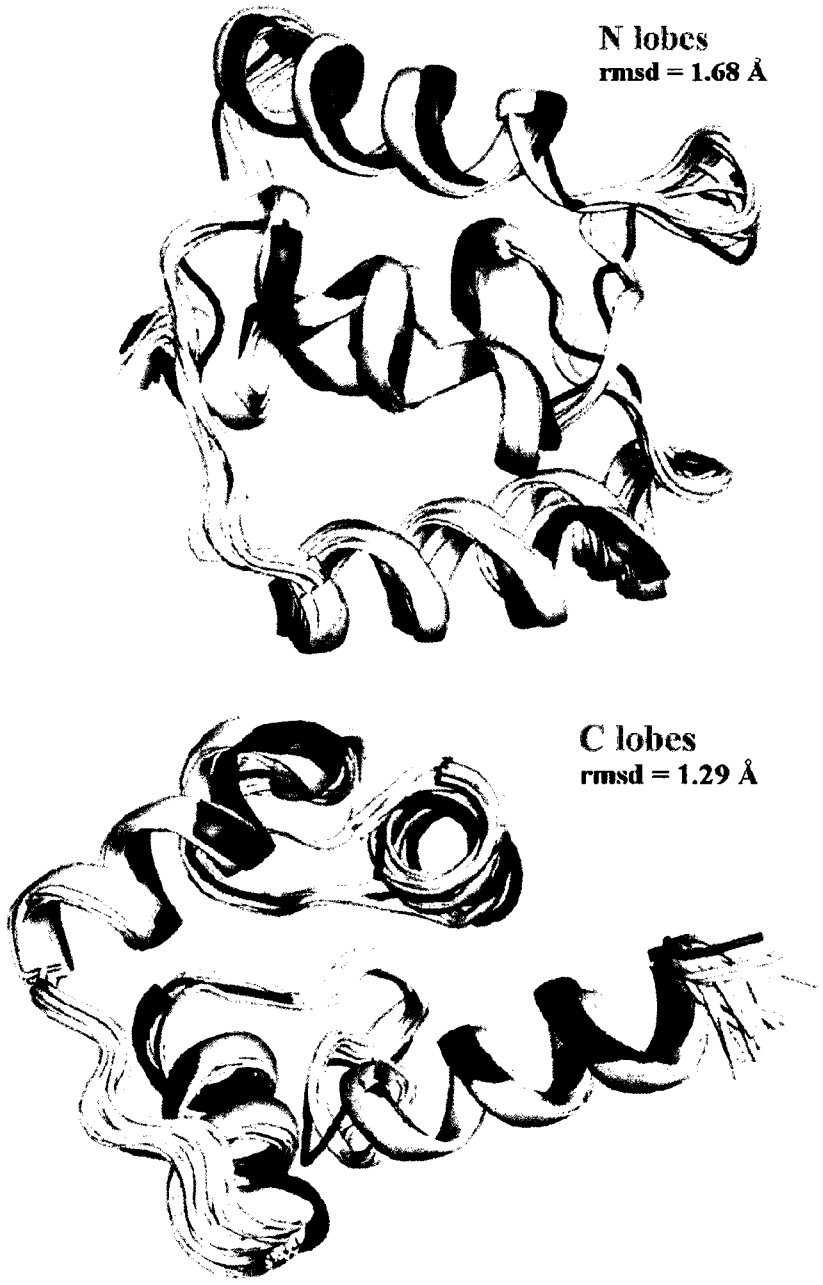


Figure 5.4 Structural comparison of holo and apoN androcam

Superposition of the average structure of holo androcam onto the 20 lowest energy structures of apoN androcam; done by aligning the Cα atoms of residues 4-74 (for N lobes) and (81-147) for C lobes. The 'rmsd' values calculated include the loop regions.

5.2.2 Addition of calcium ions in silico during ARIA calculations

Calcium ions (atomic mass number of 40) have a spin quantum number I=0, are NMR inactive and therefore provide no data to experimentally determine their spatial position relative to the liganding side chains of the protein. The positions of the side chains is decided by NOE, backbone and/or side chain dihedral angle and J coupling data only. I added calcium ions in my ARIA calculations as a two step procedure. First, I calculated the structures without including Ca^{+2} ions to determine if NMR data alone would help orient the side chains of EF hands towards a potential ligand binding pocket. Next, I added Ca^{+2} ions in silico by tying them to O^{-} atoms of liganding residues using distance restraints, and observed the effect on energies and violations. The restraint statements used for tying a Ca^{+2} ions to the 3rd and 4th EF hands are shown in Appendix C.2. The distances are derived from the 3rd and 4th EF hands of Drosophila calmodulin (Taylor et al., 1991). The energy penalty is parabolic with error bounds of 0.0 Å for each restraint. For both apoN and holo structures, the side chains of the canonical motif 'DxDxDxXxxxxE' in both lobes did not orient towards any binding pocket and sampled different positions in each structure (Figure 5.5 A). However, restricting the side chains of EF hand 3 and 4 to a calcium ion each in canonical fashion, gave very minimal changes in the energies and violations from the no Ca^{+2} added structures. The backbone of the EF hand binding loop is more clearly defined when Ca^{+2} ion is tied than without Ca^{+2} where three out of twenty structures sample an alternate conformation around D95,G96 and D97. The position of the liganding side chains in the Ca^{+2} added structures still showed some variance (Figure 5.5 B) indicative of the innate flexible nature of the loops which is consistent with comparatively less available NOE data in the loop regions.

Energies

Total	:	1269.0 kcal/mol
NOE	:	75.6 kcal/mol
dihedral	:	5.0 kcal/mol
coupling	:	83.8 kcal/mol

rms deviations

NOE	:	0.017 Å
dihedrals	:	0.670 Å
couplings	:	0.775 Å

Average violations per structure

NOE	:	0.1
dihedrals	:	0.0
couplings	:	21.4

Energies

Total	:	1269.0 kcal/mol
NOE	:	87.7 kcal/mol
dihedral	:	5.2 kcal/mol
coupling	:	72.4 kcal/mol

rms deviations

NOE	:	0.018 Å
dihedrals	:	0.668 Å
couplings	:	0.721 Å

Average violations per structure

NOE	:	0.1
dihedrals	:	0.0
couplings	:	17.4

Figure 5.5 Ca^{+2} ions in ARIA calculations of apoN androcam

Superposition of the 20 lowest energy structures of apoN androcam showing the calcium binding loop of EF hand 3 in the C lobe. Calcium ion is shown as green dot. Side chain of calcium liganding residues of D93, D95, D97, F99 and E104 are shown in sticks representation. Hydrogen atoms are excluded for clarity purpose.
A) Structures solved without Ca^{+2} ions during ARIA calculations.
B) Structures solved by including Ca^{+2} ions during ARIA calculations.

5.2.3 Comparing structures of androcam and calmodulin

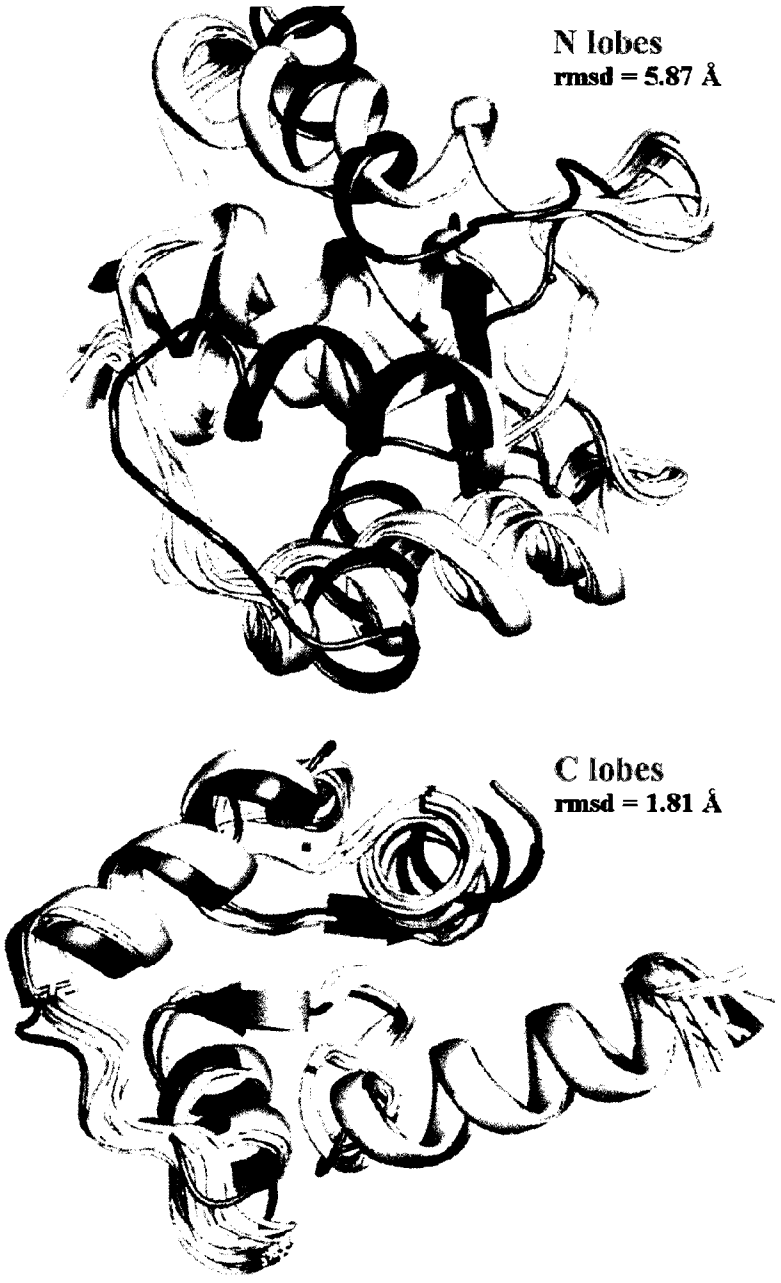
I compared my androcam structures with that of holo (Ca^{+2} bound) calmodulin (Chattopadhyaya et al., 1992) with the objective of identifying structural differences and similarities between androcam and the protein from which it has been derived by evolution. From Figure 5.6, it can be seen that the C-lobe of androcam is very similar to the C-lobe of holo calmodulin (backbone rmsd = 1.81 Å) but the N-lobes are very different from each other (backbone rmsd = 5.87 Å). However, the N-lobe of androcam superimposes well with the N-lobe of apo (Ca^{+2} free) calmodulin: note in Figure 5.7 how the four helices of the N-lobe in both structures orient as a parallel helical bundle. The 'apo' and 'holo' conformations of the lobes of calmodulin are also referred to as the 'closed' form (parallel orientation of helices) and 'open' form (perpendicular orientation of helices) respectively. The androcam structures show that the C-lobe adopts a canonical 'open' conformation whereas the N-lobe adopts a 'closed' conformation in a wide range of [Ca^{+2}] (11μM to 10mM).

The apo-like N-lobe seen for androcam at either low or high calcium can explain the biophysical experiments of (Martin et al., 1999) (K_D > 80μM) and the weak NMR shift changes seen in my Ca^{+2} titration data (K_D = ~4.1 mM) (Chapter 3.3). Androcam binds Ca^{+2} very weakly because it does not undergo the usual EF hand conformational change – and without this dramatic change, the chemical shift differences are small. Why the N-lobe cannot undergo the Ca^{+2} induced switch is not clear: certainly the mutations to key coordinating residues (Figure 1.7) are important, but these and other mutations may also help to stabilize the 'closed' state. This question could be addressed by exchanging

point mutations between androcam and calmodulin and determining the effects on Ca^{+2} affinity and structure.

The C-lobe of androcam binds Ca^{+2} much more tightly than calmodulin (Martin et al., 1999) despite having canonical liganding residues (Figure 1.7) in an 'open' fold that very closely resembles the calmodulin C-lobe. Because there is no evidence in our structures of additional coordinating protein atoms that might directly enhance Ca^{+2} affinity, I propose that non-conserved androcam C-lobe residues indirectly enhance Ca^{+2} affinity by stabilizing the 'open' state (or by destabilizing alternate states). This proposal could be tested by swapping residues between androcam and calmodulin and determining the effects on Ca^{+2} affinity. One prediction that follows from this model is that the androcam C-lobe would tend to adopt the 'open' conformation even in the absence of Ca^{+2}. This prediction is consistent with the 2D ^{1}H-^{15}N HSQC spectra of androcam in the presence of excess EDTA, which are considerably better dispersed than spectra of apo-calmodulin (not shown). In the absence of chemical shift assignments for apo-androcam, this argument is unfortunately somewhat weak; showing a bias in apo-androcam towards holo-androcam shifts would be very strong support for this idea.

Taken together, our structural data indicate that androcam is a specialist that adopts a unique calmodulin-like fold in each of its lobes and maintains this conformation over any physiological calcium fluctuations. In evolving from the archetypal calmodulin sequence, androcam has lost the ability to respond to calcium as a switch by modifying its C-lobe to be 'open' at any [Ca^{+2}] and modifying its N-lobe to remain constitutively 'closed'. This specialized calmodulin variant has lost many calmodulin-like properties; we wondered if it might also recognize myosin VI in a novel way.



Pink: apoN androcam (20 structures)
Black: holo Calmodulin (1cll.pdb)

Figure 5.6 Structural comparison of holo-calmodulin and apoN androcam

Superposition of the structure of *H.sapiens* holo-calmodulin (Chattopadhyaya et al., 1992) onto the 20 lowest energy structures of apoN androcam using the Cα atoms of residues 4-74 for N lobes and 81-147 for C lobes. The 'rmsd' values include the loop regions. Ca^{+2} ions present in the holo-calmodulin structure are shown as green dots.

Figure 5.7 Structural comparison of apo-calmodulin and apoN androcam

Superposition of the structure of *Xenopus* apo-calmodulin (Zhang et al., 1995) onto the 20 lowest energy structures of apoN androcam using the Cα atoms of residues 4-74 for N lobes and 81-147 for C lobes. The rmsd values include the loop regions.

5.2.4 Androcam and calmodulin interact differently with *Drosophila* myosin VI

It is known that androcam (and not calmodulin) co-localizes with myosin VI in *D. melanogaster* testis (Frank et al., 2006). Thus, the individualization process may require that myosin VI interacts with its light chain partner in a manner different from how it interacts with calmodulin. The specialized structure of androcam may very well provide this alternative binding partner. Myosin VI contains a unique light chain target peptide "Insert2" which when bound to Ca^{+2}-calmodulin makes specific interactions with the converter domain that reorient the lever arm by 120° causing the reversal in directionality (Menetrey et al., 2005). I performed NMR titrations of androcam and calmodulin with the Insert2 peptide to determine how binding of the peptide influenced the chemical shifts of the two proteins. I acquired ^{15}N HSQC spectra for both proteins first in the absence and then in the presence of an equimolar quantity of Insert2; results are shown in Figure 5.8. From peak movement it can be inferred that androcam interacts with Insert2 through its C-lobe only, whereas calmodulin interacts with the target peptide with both lobes. This finding explains some puzzling peptide affinity data (Martin et al., 1999) in which calmodulin binds more tightly than androcam to target peptides under biological $[Ca^{+2}]$. The tighter binding by calmodulin results from its using both lobes to bind the peptide, presumably in a BAA mode, whereas androcam binds with only one lobe. It seems likely that androcam could bind intact myosin VI in its biological context using its canonical 'open' C-lobe to bind at the peptide motif but using its non-canonical 'closed' N-lobe to contact other parts of myosin VI, or perhaps even another protein partner at the actin cones that support spermatogenesis. It would be interesting to know if the unique fold of androcam has evolved to perform a function that calmodulin cannot or if androcam binds

myosin VI in a manner that calmodulin can bind but does so independent of [Ca^{+2}]; at present, I favor the latter explanation.

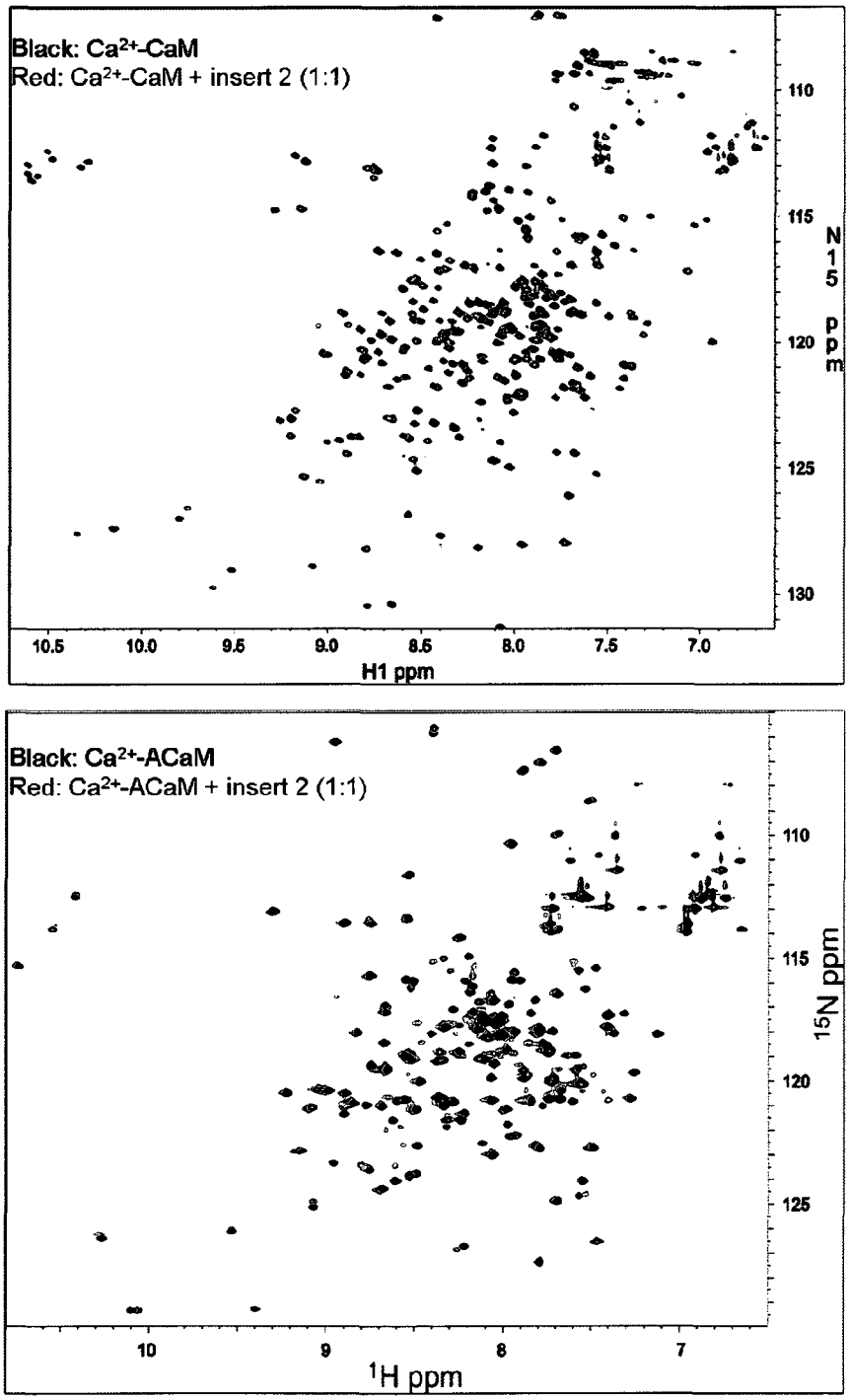


Figure 5.8 ^{15}N HSQC spectra reveal different binding modes with myosin VI

^{15}N HSQC spectra for androcam and calmodulin with (red peaks) and without (black) the Insert2 peptide. Titration is done by adding equimolar quantities of Insert2 peptide in 1:1 ratio.

5.2.5 The structure of androcam is one of the binding modes adopted by calmodulin

Because structures are available for dozens of calmodulin/target complexes, I examined these structures to seek commonalities and contrasts with androcam. The pathogenic bacterium *Bacillus anthracis* (anthrax) releases an exotoxin, edema factor, that is responsible for increasing the concentration of cyclic AMPs in affected cells (Ladant and Ullmann, 1999; Leppla, 1982). Edema factor is activated by the endogenous and ubiquitously present calmodulin (Eldik and Watterson, Academic Press New York, 1998). Calmodulin binds edema factor with a Ca^{+2} saturated 'open' C-lobe and a Ca^{+2}-free 'closed' N-lobe (see Figure 5.9) (Drum et al., 2002). The N-lobe of calmodulin bound to the anthrax toxin superimposes well onto the N lobes of the androcam apoN family of structures with a Cα rmsd of 1.66 Å (Figure 5.10), and the C-lobes also superimpose closely with a Cα rmsd of 1.91 Å.

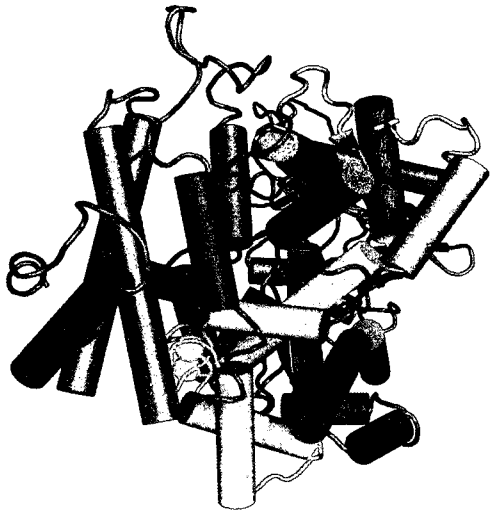
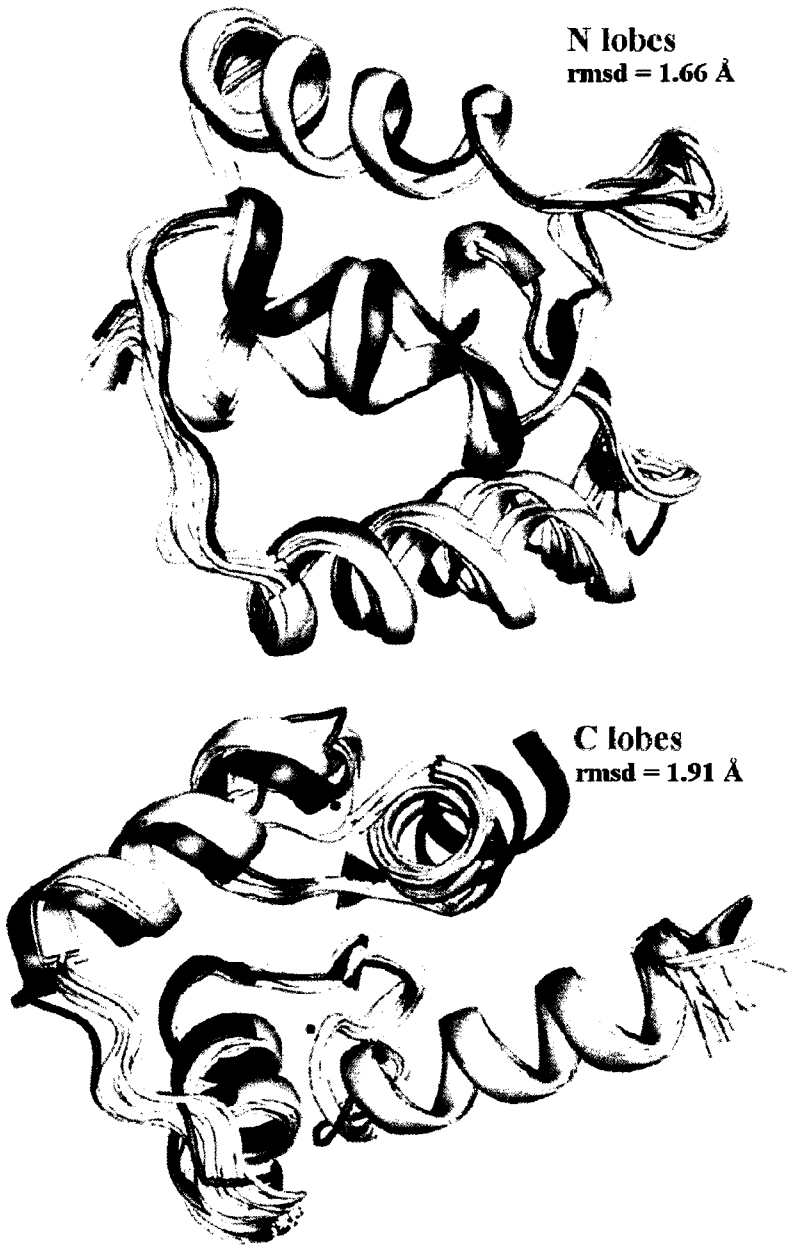


Figure 5.9 Calmodulin bound to the anthrax exotoxin edema factor

Calmodulin binds to the anthrax exotoxin 'edema factor' (black) a calmodulin activated adenylyl cyclase, with a Ca^{+2} saturated C lobe (blue) and a Ca^{+2} free N lobe (teal). 1K93 : (Drum et al., 2002).



Pink: apoN androcam (20 structures)
Teal: Calmodulin bound to anthrax exotoxin edema factor (1K93.pdb)

Figure 5.10 Structure of androcam is a special binding mode adopted by calmodulin

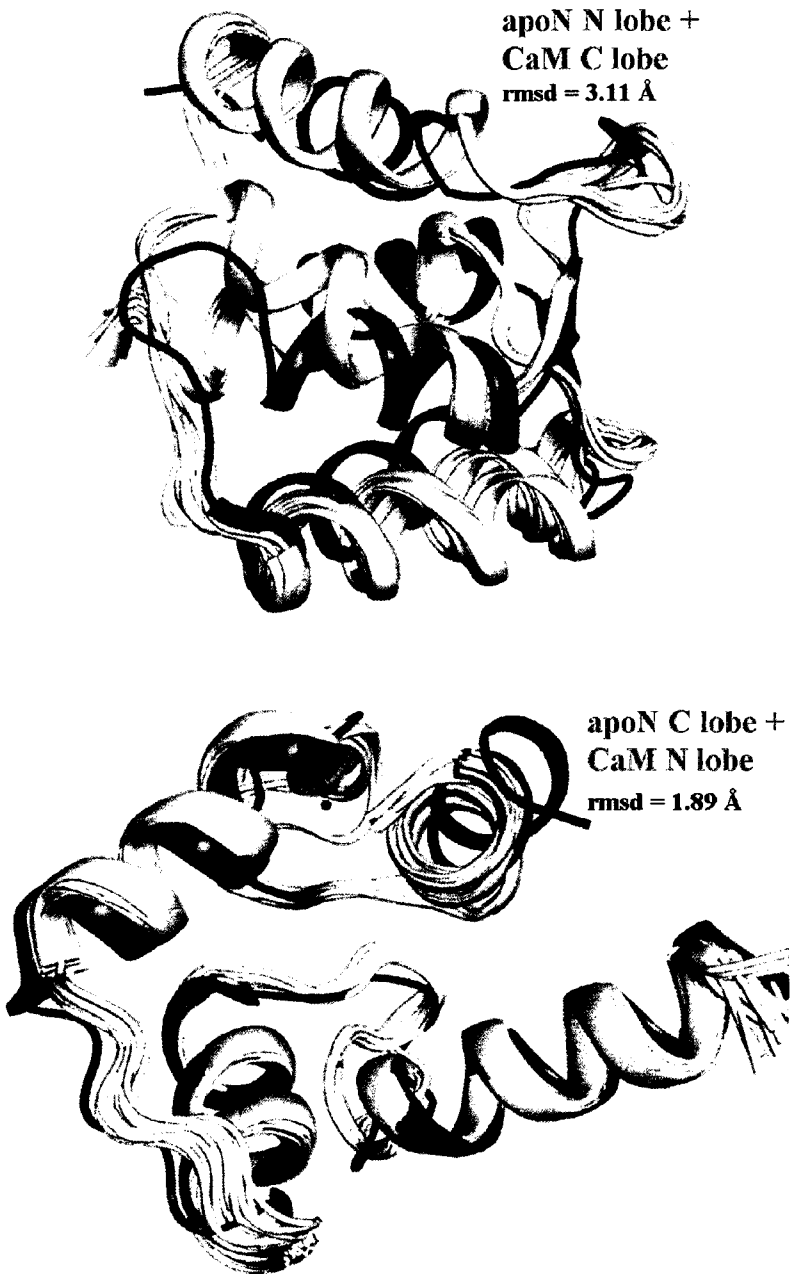
Superposition of the structure of calmodulin bound to the anthrax exotoxin edema factor (Drum et al, 2002) onto the 20 lowest energy structures of apoN androcam using the Cα atoms of residues 4-74 (for N lobes) and (81-147) for C lobes. The rmsd values include the loop regions. Ca^{+2} ions bound to C lobe of calmodulin are shown as green dots.

Thus the single, constitutive structure adopted by androcam corresponds closely to one of the many binding modes of calmodulin; this particular conformation of calmodulin uses its C-lobe to bind a hydrophobic anchor in an 'open' fashion but uses the outer surface of its N-lobe (rather than a pocket) to contact the edema factor. Given that my 'Insert2' titration data has already established that androcam binds to the myosin VI target peptide with its C-lobe only, it is tantalizing to speculate that the androcam N-lobe interacts with some other region of myosin VI (or with some other binding partner) in the mode seen in the edema factor structure. It would also be interesting to know if calmodulin can adopt this conformation on this target at all, and especially under the calcium concentrations found in the *D. melanogaster* testis. I hypothesize that calmodulin may adopt an androcam-like open C lobe and a closed N lobe in the late recovery process reconstructing properly progressing actin cones in the androcam RNAi knockout flies (see Figure 1.5).

5.2.6 Structural variant of the "closed" form

In contrast to what is seen with the edema factor bound structure, calmodulin is also known to adopt a Ca^{+2}-loaded 'open' N-lobe and a Ca^{+2}-free 'closed' C-lobe when bound to the small conductance K^+ channel (SK channel) (Schumacher et al., 2001). The calmodulin binding domain (CaMBD) of the SK channel is a dimer of two α-helices. Two molecules of calmodulin bind to two CaMBD dimers with each calmodulin molecule contacting three helices. One helix contact is mediated by a Ca^{+2} bound N-lobe and the other two by a Ca^{+2} free C-lobe. The holo N-lobe of calmodulin in this structure superimposes well with the C-lobe of Ca^{+2} bound androcam (rmsd = 1.89 Å; see Figure

5.11). However, the Ca^{+2}-free C-lobe fits poorly to the N lobe of apoN androcam (rmsd = 3.11Å). It would seem that the lobes of androcam and calmodulin adopt structurally very similar open conformations but not so similar closed conformations. Whether the differences in the closed conformations are due to the protein sequence or driven by the sequence of the bound target remains to be determined.



Pink: apoN androcam (20 structures)
Purple: Calmodulin bound to SK channel (1G4Y.pdb)

Figure 5.11 Structural comparison of androcam with open and closed states of calmodulin bound to SK channels.

Calmodulin bound to the small conductance potassium channels (Schumacher et al., 2001) superimposed onto the 20 lowest energy structures of apoN androcam. The Cα atoms of residues 4-74 of the Ca^{+2} bound N-lobe of calmodulin are fit to Cα atoms of residues 81-147 of the C-lobe of apoN androcam. The Cα atoms of residues 85-147 of the Ca^{+2} free C-lobe of calmodulin are fit to Cα atoms of residues 4-80 of the N-lobe of apoN androcam. The rmsd values include the loop regions. Ca^{+2} ions bound to N-lobe of calmodulin are shown as green dots.

5.3 Remarks, or how the calmodulin variant got its hump

Given the diverse range of structures that calmodulin adopts, its sensitivity to Ca^{+2}, and the strong sequence identity between androcam and calmodulin (67%), it might have been hard to distinguish between androcam and calmodulin. Although the C-lobes of androcam and calmodulin cannot be distinguished based on their structures, the N-lobes of these proteins are very different indeed, and it is clear that androcam is essentially unresponsive to Ca^{+2} despite this being one of the key functions of the ancient sequence from which it has evolved. What lessons can we take from these findings?

One important clue to this riddle is the incredible sequence conservation of calmodulin across species. Vertebrates have one calmodulin sequence – no variations are tolerated, even the slightest sequence drift, perhaps because the archetypal calmodulin sequence is poised so that it does not overly favor any one of the many possible states that calmodulin can adopt. Favoring one fold or binding mode too strongly would skew many regulatory events, so the sequence of calmodulin is highly constrained.

How then might a sequence variant such as androcam have arisen? Suppose that a duplicate copy of calmodulin were placed under control of a testis-specific promoter in an ancestral arthropod. If a biological process linked to evolutionary fitness (such as spermatogenesis) were favorably influenced by the presence of elevated calmodulin, then the duplicated gene would fix in the population. Although the original, ubiquitously expressed copy of calmodulin would still experience strong selective pressure to maintain its sequence because of the many roles it plays throughout the organism, the copy that was now tissue-specifically expressed would experience selective pressures related only to the effects of gene function in that one tissue. Because calmodulin is also expressed in

this tissue, the duplicate copy would be almost redundant, and sequence changes in the second copy that did not affect the additional evolutionary fitness imparted via that tissue would be effectively silent. If the selective advantage were the result of interactions with many diverse target molecules in the tissue, then the sequence of the second copy would also be highly constrained. But if the selective advantage occurred primarily because of interactions with just one other protein, the sequence of the second copy of calmodulin would be free either to drift or to optimize interaction with that target. If this protein were a player in some evolutionary molecular arms race, such as the production of sperm that outcompete those of other males, then selective pressures would rapidly change the sequence of the calmodulin variant.

Although the origins of the spectacular spermatogenesis of Drosophilidae will remain shrouded in mystery, it is known that a gene duplication event produced a testis-specific copy of calmodulin enhancing myosin VI function in spermatogenesis and setting off competitive changes that altered the development and morphology of this tissue. Although such a tale could of course occur for any duplicated gene, it must be noted that the structural plasticity and binding versatility of the archetypal calmodulin gene make it already capable of sampling many states. Rather than needing to evolve a new binding mode from scratch, which is rather improbable, the sequence could incrementally be improved by mutations that favor the key conformation/ target binding or disfavoring any conformations not relevant to its specialized function in this tissue. The pluripotential nature of the calmodulin sequence suggests that many calmodulin-like proteins represent structural and functional specialization following gene duplication events; the open question is, which structures and functions are retained?

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APPENDIX A

A.1 Ca^{+2} BINDING TO ANDROCAM (TWO STATE MODEL)

Consider Ca^{+2} binding to a single site on the N-lobe of androcam having a Ca^{+2} saturated C-lobe,

$$A + C \leftrightarrow AC$$

where A: androcam, C: calcium ion, AC: androcam-calcium complex

from mass balance, the total calcium in excess of a fully saturated C lobe is given as,

$$C_T = [C] + [AC] \quad \text{..... (1)}$$

$$A_T = [A] + [AC] \quad \text{..... (2)}$$

where [C] : free calcium in solution, [A] : free androcam in solution, A_T : total concentration of androcam (C-lobe fully Ca^{+2} loaded).

The binding (dissociation) constant for the above reaction is given as,

$$K_D = \frac{[A]\cdot[C]}{[AC]} \quad \text{upon re-arranging} \quad [A] = \frac{K_D \cdot [AC]}{[C]} \quad \text{..... (3)}$$

The fraction of androcam free of Ca^{+2} can be defined as,

$$Y = \frac{[A]}{A_T} \quad \text{..... (4)}$$

Substituting from Eqn.(3) and Eqn.(2) ,

$$Y = \frac{K_D\,[AC]}{A_T \cdot (C_T - [AC])} \quad \text{..... (5)}$$

Dividing by Eqn.(2) by A_T and re-arranging,

$$A_T \cdot (1-Y) = [AC] \quad \text{..... (6)}$$

Combining Eqn.(5) and Eqn.(6) ,

$$Y = \frac{K_D\,A_T\,(1-Y)}{A_T\,[C_T - A_T\,(1-Y)]}$$

i.e. $A_T Y^2 + (C_T - A_T + K_D)Y - K_D = 0$

Solving the quadratic equation for Y,

$$Y = \frac{A_T - C_T - K_D \pm \sqrt{(C_T - A_T + K_D)^2 + 4 A_T K_D}}{2 A_T} \quad \text{..... (7)}$$

Also,

$$Y = \frac{[A]}{A_T} = \frac{K_D([AC])}{A_T([C])} = \left(\frac{K_D}{A_T}\right)\left(\frac{A_T(1-Y)}{[C]}\right)$$

$$Y = \frac{1}{1 + \left(\frac{[C]}{K_D}\right)} \quad \text{..... (8)}$$

A.2 Ca^{+2} BINDING TO ANDROCAM IN PRESENCE OF BUFFER (TWO STATE MODEL)

Consider Ca^{+2} binding to a single site on the N-lobe of androcam having a Ca^{+2} saturated C-lobe in the presence of a buffer (K^{+}Citrate),

$$A + C \leftrightarrow AC$$

$$B + C \leftrightarrow BC$$

where A: androcam, C: calcium ion, AC: androcam-calcium complex
where B: pottasium citrate, C: calcium ion, BC: citrate-calcium complex

from mass balance, the total calcium, androcam and citrate is given as,

$$C_T = [C] + [AC] + [BC] \quad \text{..... (1)}$$

$$A_T = [A] + [AC] \quad \text{..... (2)}$$

$$B_T = [B] + [BC] \quad \text{..... (3)}$$

where [C] : free calcium in solution, [A] : free androcam in solution,
[B] : free buffer in solution,
A_T : total concentration of androcam (C-lobe fully Ca^{+2} loaded).

The binding (dissociation) constant for the above reaction is given as,

$$K_D = \frac{[A]\cdot[C]}{[AC]} \quad \text{upon re-arranging} \quad [A] = \frac{K_D \cdot [AC]}{[C]} \quad \text{..... (4)}$$

$$K_B = \frac{[B]\cdot[C]}{[BC]} \quad \text{upon re-arranging} \quad [B] = \frac{K_B \cdot [BC]}{[C]} \quad \text{..... (5)}$$

The fraction of androcam free of Ca^{+2} can be defined as,

$$Y = \frac{[A]}{A_T} \quad \text{..... (6)}$$

Substituting Eqn.(4) into Eqn.(2) and Eqn.(5) into Eqn.(3) we get,

$$A_T = \frac{K_D\,[AC]}{[C]} + [AC] \qquad B_T = \frac{K_B\,[BC]}{[C]} + [BC]$$

Upon rearranging,

$$[BC] = \frac{B_T[C]}{[C]+K_B} \qquad [AC] = \frac{A_T[C]}{[C]+K_D}$$

Substituting in Eqn.(1),

$$C_T = [C] + \frac{A_T[C]}{[C]+K_D} + \frac{B_T[C]}{[C]+K_B}$$

Upon rearranging,

$$[C]^3 + \left(K_B + K_D + A_T + B_T - C_T\right)\cdot[C]^2 +$$
$$\left(K_D K_B + A_T K_B + B_T K_D - C_T K_B - C_T K_D\right)\cdot[C] - K_D K_B C_T = 0$$

..... (6)

Also,

$$Y = \frac{[A]}{A_T} = \frac{K_D([AC])}{A_T([C])} = \left(\frac{K_D}{A_T}\right)\left(\frac{A_T(1-Y)}{[C]}\right)$$

$$Y = \frac{1}{1+\left(\frac{[C]}{K_D}\right)} \qquad \text{..... (8)}$$

APPENDIX B (holo androcam)

B.1 Chemical shifts for holo androcam

Group	Atom	Shift
S2	CA	57.93
S2	CB	63.04
S2	HA	4.35
S2	HB#	3.80
E3	HN	8.57
E3	N	121.80
E3	CA	55.62
E3	CB	29.47
E3	CG	35.47
E3	HA	4.29
E3	HB2	1.89
E3	HB3	2.05
E3	HG2	2.23
E3	HG3	2.26
L4	HN	8.19
L4	N	121.22
L4	CA	53.33
L4	CB	42.53
L4	CG	26.22
L4	CD1	25.61
L4	CD2	22.62
L4	HA	4.58
L4	HB2	1.28
L4	HB3	1.66
L4	HD1#	0.88
L4	HD2#	0.82
L4	HG	1.67
T5	HN	8.75
T5	N	113.45
T5	CA	59.41
T5	CB	70.30
T5	CG2	21.08
T5	HA	4.42
T5	HB	4.74
T5	HG2#	1.29
E6	HN	8.98
E6	N	120.33
E6	CA	59.02
E6	CB	28.45
E6	CG	35.50
E6	HA	3.90
E6	HB#	2.01
E6	HG2	2.31
E6	HG3	2.37
E7	HN	8.64
E7	N	119.44
E7	CA	59.28
E7	CB	28.09
E7	CG	36.05
E7	HA	4.02
E7	HB2	1.90
E7	HB3	2.00
E7	HG2	2.33
E7	HG3	2.41
Q8	HN	7.67
Q8	N	120.66
Q8	CA	57.79
Q8	CB	28.24
Q8	CG	33.95
Q8	HA	3.75
Q8	HB2	1.18
Q8	HB3	2.14
Q8	HG2	2.19
Q8	HG3	2.26
Q8	NE2	111.00
Q8	HE21	6.65
Q8	HE22	7.61
I9	HN	8.09
I9	N	118.14
I9	CD1	11.84
I9	CG2	16.71
I9	CA	64.88
I9	CB	36.75
I9	CG1	29.22
I9	HA	3.37
I9	HB	1.83
I9	HG12	1.00
I9	HG13	1.61
I9	HD1#	0.76
I9	HG2#	1.11
A10	HN	7.84
A10	N	120.75
A10	CA	54.51
A10	HA	4.11
A10	HB#	1.48
A10	CB	17.15
E11	HN	7.72
E11	N	119.80
E11	CA	58.44
E11	CB	28.61
E11	CG	35.34
E11	HA	4.16
E11	HB#	2.15
E11	HG2	2.29
E11	HG3	2.48
F12	HN	9.04
F12	N	120.27
F12	CA	56.75
F12	CB	35.74
F12	HA	4.90
F12	HB2	3.58
F12	HB3	3.92
F12	HD#	7.21
F12	HE#	7.30
F12	HZ	7.13
K13	HN	9.14
K13	N	122.78
K13	CA	58.97
K13	HA	3.70
K13	CB	30.56
K13	HB2	1.65
K13	HB3	1.93
K13	HG2	0.78
K13	HG3	1.18
K13	CG	23.39
K13	HD2	1.05
K13	HD3	1.20
K13	CD	27.34
K13	CE	40.76
K13	HE#	2.51
D14	HA	4.40
D14	HN	7.80
D14	N	117.88
D14	CB	39.91
D14	CA	56.41
D14	HB2	2.70
D14	HB3	2.80
A15	HN	7.55
A15	N	120.11
A15	CA	53.94
A15	HA	4.36
A15	HB#	1.64
A15	CB	18.73
F16	HN	8.88
F16	N	120.43
F16	CA	61.49
F16	CB	40.15
F16	HA	3.78
F16	HB2	3.13
F16	HB3	3.37
F16	HD#	6.73
F16	HE#	6.94
F16	HZ	7.13
V17	HN	8.54
V17	N	113.30
V17	CA	64.58
V17	CB	30.83
V17	CG1	20.41
V17	CG2	20.27
V17	HA	3.89
V17	HB	2.34
V17	HG1#	1.14
V17	HG2#	1.09
Q18	HN	7.40
Q18	N	117.75
Q18	CA	56.90
Q18	CB	27.07
Q18	CG	32.91
Q18	HA	3.93
Q18	HB2	1.96
Q18	HB3	2.08
Q18	HG2	2.39
Q18	HG3	2.49
Q18	NE2	112.90
Q18	HE21	6.80
Q18	HE22	7.39
F19	HN	7.68
F19	N	116.38
F19	CA	57.89
F19	CB	38.89
F19	HA	4.25
F19	HB2	2.67
F19	HB3	3.04
F19	HD#	7.30
F19	HE#	7.30
F19	HZ	7.13
D20	HA	4.91
D20	HN	7.49
D20	N	122.66
D20	CB	38.97
D20	CA	51.36
D20	HB2	2.10
D20	HB3	2.73
K21	HN	8.05
K21	N	122.89
K21	CA	57.85
K21	HA	4.03
K21	CB	31.36
K21	HB#	1.84
K21	HG2	1.42

K21	HG3	1.46	I27	HN	8.53	L32	N	119.99	R37	HN	8.24
K21	CG	24.19	I27	N	111.51	L32	CA	57.72	R37	N	118.74
K21	CD	27.87	I27	CD1	13.70	L32	CB	41.33	R37	CA	58.50
K21	CE	41.27	I27	CG2	16.72	L32	CG	26.09	R37	HA	4.60
K21	HD2	1.65	I27	CA	57.45	L32	CD1	22.42	R37	CB	29.47
K21	HD3	1.66	I27	CB	40.75	L32	CD2	25.45	R37	CG	28.32
K21	HE#	2.98	I27	CG1	24.48	L32	HA	3.67	R37	HB2	1.91
E22	HN	8.74	I27	HA	4.53	L32	HB2	1.41	R37	HB3	1.96
E22	N	115.63	I27	HB	1.56	L32	HB3	1.85	R37	HG2	1.82
E22	CA	55.35	I27	HG12	0.75	L32	HD1#	0.99	R37	HG3	1.90
E22	CB	29.22	I27	HG13	1.12	L32	HD2#	0.86	R37	CD	42.74
E22	CG	36.02	I27	HD1#	0.27	L32	HG	1.49	R37	HD2	3.08
E22	HA	4.38	I27	HG2#	-0.04	G33	HN	8.95	R37	HD3	3.25
E22	HB2	2.04	A28	HN	8.68	G33	N	106.14	T38	HN	8.13
E22	HB3	2.24	A28	N	124.31	G33	CA	47.30	T38	N	117.72
E22	HG2	2.24	A28	CA	50.54	G33	HA1	3.80	T38	CA	65.35
E22	HG3	2.29	A28	HA	4.80	G33	HA2	3.45	T38	CB	68.31
G23	HN	7.95	A28	HB#	1.55	T34	HN	7.77	T38	CG2	20.48
G23	N	110.27	A28	CB	18.50	T34	N	118.40	T38	HA	4.21
G23	CA	46.54	T29	HN	8.51	T34	CA	65.20	T38	HB	4.59
G23	HA1	3.92	T29	N	115.98	T34	CB	67.53	T38	HG2#	1.48
G23	HA2	3.87	T29	CA	64.95	T34	CG2	21.87	L39	HN	7.27
T24	HN	9.29	T29	CB	66.43	T34	HA	3.83	L39	N	120.67
T24	N	113.03	T29	CG2	23.18	T34	HB	4.16	L39	CA	53.50
T24	CA	61.41	T29	HA	3.60	T34	HG2#	1.40	L39	CB	41.91
T24	CB	69.81	T29	HB	4.15	L35	HN	7.68	L39	CG	25.97
T24	CG2	21.31	T29	HG2#	1.11	L35	N	124.77	L39	CD1	22.09
T24	HA	4.31	R30	HN	8.03	L35	CA	57.53	L39	CD2	25.84
T24	HB	4.35	R30	N	117.60	L35	CB	39.35	L39	HA	4.58
T24	HG2#	1.20	R30	CA	57.26	L35	CG	26.19	L39	HB2	1.76
G25	HN	10.73	R30	HA	4.23	L35	CD1	21.89	L39	HB3	1.88
G25	N	115.22	R30	CB	28.57	L35	CD2	26.25	L39	HD1#	1.17
G25	CA	44.58	R30	CG	26.13	L35	HA	3.67	L39	HD2#	0.98
G25	HA1	3.79	R30	HB2	1.86	L35	HB2	0.34	L39	HG	1.73
G25	HA2	4.34	R30	HB3	1.95	L35	HB3	1.43	G40	HN	7.78
K26	HN	7.73	R30	HG2	1.58	L35	HD1#	0.53	G40	N	107.00
K26	N	118.72	R30	HG3	1.64	L35	HD2#	-0.09	G40	CA	45.16
K26	CA	53.80	R30	CD	42.23	L35	HG	1.06	G40	HA1	3.82
K26	HA	5.42	R30	HD#	3.22	M36	HN	8.82	G40	HA2	4.25
K26	CB	36.18	E31	HN	7.80	M36	N	117.95	Q41	HN	7.93
K26	CD	28.60	E31	N	118.00	M36	CA	60.40	Q41	N	117.93
K26	CE	41.52	E31	CA	55.00	M36	CB	31.95	Q41	CA	52.65
K26	HB2	1.52	E31	CB	29.01	M36	CG	32.52	Q41	CB	29.43
K26	HB3	1.58	E31	CG	35.94	M36	CE	15.90	Q41	CG	32.42
K26	HD2	1.46	E31	HA	4.62	M36	HA	3.76	Q41	HA	4.42
K26	HD3	1.59	E31	HB2	1.89	M36	HB2	1.91	Q41	HB2	1.52
K26	HG2	1.22	E31	HB3	2.26	M36	HB3	2.02	Q41	HB3	2.08
K26	HG3	1.32	E31	HG2	2.25	M36	HG2	2.48	Q41	HG2	1.98
K26	CG	24.30	E31	HG3	2.35	M36	HG3	2.87	Q41	HG3	2.18
K26	HE#	2.96	L32	HN	7.56	M36	HE#	1.76	Q41	NE2	112.90

Q41	HE21	6.18	E47	CB	28.96	I52	N	116.21	N57	HD21	6.95
Q41	HE22	7.19	E47	CG	36.14	I52	CD1	12.92	N57	HD22	7.72
N42	HN	8.65	E47	HA	4.01	I52	CG2	16.77	N58	HN	8.11
N42	N	117.15	E47	HB2	1.87	I52	CA	63.69	N58	N	117.50
N42	CA	50.17	E47	HB3	2.29	I52	CB	37.65	N58	CA	53.08
N42	CB	38.60	E47	HG2	2.25	I52	CG1	27.53	N58	CB	38.83
N42	HA	5.17	E47	HG3	2.35	I52	HA	3.70	N58	HA	4.80
N42	HB2	2.74	L48	HN	8.48	I52	HB	1.90	N58	HB2	2.80
N42	HB3	2.51	L48	N	120.00	I52	HG12	1.04	N58	HB3	2.86
N42	ND2	112.50	L48	CA	57.23	I52	HG13	1.58	N58	ND2	113.90
N42	HD21	6.73	L48	CB	41.03	I52	HD1#	0.78	N58	HD21	6.95
N42	HD22	7.50	L48	CG	26.38	I52	HG2#	0.84	N58	HD22	7.72
P43	N	109.60	L48	CD#	23.80	A53	HN	7.80	N59	N	117.50
P43	CA	61.54	L48	HA	3.93	A53	N	122.62	N59	CA	52.60
P43	CB	30.71	L48	HB#	1.66	A53	CA	54.46	N59	CB	38.67
P43	CG	26.26	L48	HD#	0.81	A53	HA	4.11	N59	HA	4.89
P43	CD	49.23	L48	HG	1.60	A53	HB#	1.52	N59	HB2	2.80
P43	HA	4.72	Q49	HN	8.05	A53	CB	17.46	N59	HB3	3.17
P43	HB2	1.96	Q49	N	116.63	E54	HN	8.03	N59	ND2	113.00
P43	HB3	2.05	Q49	CA	58.04	E54	N	117.51	N59	HD21	6.91
P43	HG2	1.91	Q49	CB	27.05	E54	CA	57.88	N59	HD22	7.71
P43	HG3	1.98	Q49	CG	32.94	E54	CB	28.50	N60	HN	8.66
P43	HD2	3.23	Q49	HA	3.89	E54	CG	35.34	N60	N	116.92
P43	HD3	3.66	Q49	HB2	2.12	E54	HA	4.10	N60	CA	53.12
T44	HN	8.90	Q49	HB3	2.16	E54	HB#	2.07	N60	CB	37.41
T44	N	113.53	Q49	HG#	2.48	E54	HG2	2.26	N60	HA	4.62
T44	CA	60.05	Q49	NE2	112.40	E54	HG3	2.34	N60	HB2	2.76
T44	CB	69.94	Q49	HE21	6.83	A55	HN	7.93	N60	HB3	3.02
T44	CG2	21.04	Q49	HE22	7.55	A55	N	122.17	N60	ND2	112.50
T44	HA	4.37	D50	HA	4.48	A55	CA	52.97	N60	HD21	6.87
T44	HB	4.70	D50	HN	7.56	A55	HA	4.30	N60	HD22	7.55
T44	HG2#	1.35	D50	N	119.44	A55	HB#	1.40	G61	HN	8.39
E45	HN	8.85	D50	CB	39.72	A55	CB	17.91	G61	N	105.68
E45	N	120.83	D50	CA	56.42	E56	HN	8.54	G61	CA	45.62
E45	CA	59.16	D50	HB2	2.69	E56	N	118.87	G61	HA1	3.76
E45	CB	28.23	D50	HB3	2.74	E56	CA	57.78	G61	HA2	4.13
E45	CG	35.36	L51	HN	8.06	E56	CB	28.40	Q62	HN	7.60
E45	HA	4.00	L51	N	120.71	E56	CG	36.07	Q62	N	118.89
E45	HB#	2.03	L51	CA	56.91	E56	HA	3.99	Q62	CA	53.40
E45	HG2	2.31	L51	CB	41.59	E56	HB2	2.06	Q62	CB	32.64
E45	HG3	2.35	L51	CG	25.63	E56	HB3	2.11	Q62	CG	32.90
A46	HN	8.33	L51	CD1	24.22	E56	HG2	2.25	Q62	HA	5.34
A46	N	120.90	L51	CD2	22.00	E56	HG3	2.35	Q62	HB2	1.91
A46	CA	54.16	L51	HA	4.06	N57	HN	8.11	Q62	HB3	1.94
A46	HA	4.07	L51	HB2	1.29	N57	N	117.50	Q62	HG2	2.18
A46	HB#	1.37	L51	HB3	2.03	N57	CA	53.61	Q62	HG3	2.31
A46	CB	17.49	L51	HD1#	0.75	N57	CB	38.40	Q62	NE2	111.40
E47	HN	7.75	L51	HD2#	0.87	N57	HA	4.67	Q62	HE21	6.76
E47	N	118.86	L51	HG	1.92	N57	HB#	2.78	Q62	HE22	7.34
E47	CA	57.95	I52	HN	8.17	N57	ND2	113.60	L63	HN	9.08

L63	N	121.04	F68	N	120.92	A73	CB	17.14	E78	HN	8.34
L63	CA	52.84	F68	CA	60.65	K74	HN	7.40	E78	N	121.60
L63	CB	45.29	F68	CB	38.32	K74	N	117.34	E78	CA	56.18
L63	CG	25.26	F68	HA	4.09	K74	CA	58.27	E78	CB	29.17
L63	CD1	24.71	F68	HB#	3.18	K74	HA	3.97	E78	CG	35.11
L63	CD2	26.71	F68	HD#	6.96	K74	CB	31.59	E78	HA	4.34
L63	HA	5.00	F68	HE#	7.23	K74	HB2	1.87	E78	HB2	1.99
L63	HB#	1.65	F68	HZ	7.13	K74	HB3	1.91	E78	HB3	2.12
L63	HD1#	0.85	C69	HN	8.32	K74	HG2	1.37	E78	HG2	2.28
L63	HD2#	0.86	C69	N	117.72	K74	HG3	1.50	E78	HG3	2.36
L63	HG	1.65	C69	CA	64.24	K74	CG	24.34	T79	HN	8.21
N64	HN	9.22	C69	CB	25.66	K74	HD#	1.62	T79	N	116.03
N64	N	120.42	C69	HA	3.35	K74	CD	28.64	T79	CA	61.61
N64	CA	50.07	C69	HB2	2.33	K74	CE	41.15	T79	CB	68.89
N64	CB	38.41	C69	HB3	2.79	K74	HE#	2.90	T79	CG2	20.89
N64	HA	5.66	G70	HN	7.69	Q75	HN	8.01	T79	HA	4.31
N64	HB2	2.78	G70	N	106.44	Q75	N	118.80	T79	HB	4.25
N64	HB3	3.50	G70	CA	46.22	Q75	CA	56.44	T79	HG2#	1.23
N64	ND2	110.70	G70	HA1	3.74	Q75	CB	27.33	D80	HA	4.75
N64	HD21	6.91	G70	HA2	3.81	Q75	CG	32.20	D80	HN	8.53
N64	HD22	7.45	I71	HN	7.55	Q75	HA	4.01	D80	N	123.85
F65	HN	8.66	I71	N	124.03	Q75	HB2	2.02	D80	CB	40.32
F65	N	118.35	I71	CD1	14.01	Q75	HB3	2.06	D80	CA	53.95
F65	CA	61.16	I71	CG2	16.52	Q75	HG#	2.30	D80	HB2	2.77
F65	CB	38.25	I71	CA	64.39	Q75	NE2	110.00	D80	HB3	2.72
F65	HA	3.62	I71	CB	37.48	Q75	HE21	6.76	T81	HN	8.33
F65	HB2	2.33	I71	CG1	28.75	Q75	HE22	7.36	T81	N	114.85
F65	HB3	2.66	I71	HA	3.59	M76	HN	8.03	T81	CA	62.23
F65	HD#	6.61	I71	HB	1.81	M76	N	116.81	T81	CB	69.13
F65	HE#	6.80	I71	HG12	1.07	M76	CA	56.27	T81	CG2	20.98
F65	HZ	7.53	I71	HG13	1.71	M76	CB	32.55	T81	HA	4.32
T66	HN	8.12	I71	HD1#	0.82	M76	CG	31.27	T81	HB	4.44
T66	N	117.48	I71	HG2#	0.80	M76	CE	16.00	T81	HG2#	1.29
T66	CA	66.12	M72	HN	7.72	M76	HA	4.20	E82	HN	8.50
T66	CB	67.48	M72	N	117.95	M76	HB2	2.03	E82	N	122.58
T66	CG2	21.04	M72	CA	55.18	M76	HB3	2.22	E82	CA	58.33
T66	HA	3.60	M72	CB	29.52	M76	HG2	2.54	E82	CB	28.55
T66	HB	4.20	M72	HA	3.97	M76	HG3	2.68	E82	CG	35.33
T66	HG2#	1.22	M72	HB2	1.41	M76	HE#	2.10	E82	HA	4.18
E67	HN	8.75	M72	HB3	1.52	R77	HN	7.63	E82	HB#	2.15
E67	N	123.51	M72	CE	15.98	R77	N	120.04	E82	HG2	2.29
E67	CA	58.08	M72	HE#	1.53	R77	CA	56.39	E82	HG3	2.47
E67	CB	29.05	M72	CG	31.55	R77	HA	4.27	E83	HN	8.37
E67	CG	36.04	M72	HG2	0.93	R77	CB	29.81	E83	N	118.95
E67	HA	3.98	M72	HG3	1.04	R77	CG	26.32	E83	CA	58.80
E67	HB2	1.87	A73	HN	8.36	R77	HB#	1.91	E83	CB	28.54
E67	HB3	2.36	A73	N	120.60	R77	HG2	1.68	E83	CG	35.60
E67	HG2	2.26	A73	CA	54.36	R77	HG3	1.73	E83	HA	4.02
E67	HG3	2.49	A73	HA	3.99	R77	CD	42.68	E83	HB2	2.05
F68	HN	8.68	A73	HB#	1.42	R77	HD#	3.20	E83	HB3	2.10

E83	HG2	2.32	A88	HB#	1.79	D93	N	116.60	F99	HE#	7.45
E83	HG3	2.36	A88	CB	17.08	D93	CB	37.47	F99	HZ	6.99
E84	HN	8.12	F89	HN	8.52	D93	CA	51.26	I100	HN	10.26
E84	N	119.06	F89	N	118.95	D93	HB2	1.42	I100	N	126.28
E84	CA	58.55	F89	CA	61.24	D93	HB3	2.31	I100	CD1	15.14
E84	CB	28.66	F89	CB	38.60	R94	HN	7.56	I100	CG2	17.20
E84	CG	35.60	F89	HA	3.09	R94	N	124.60	I100	CA	59.67
E84	HA	4.13	F89	HB2	3.05	R94	CA	57.99	I100	CB	37.97
E84	HB2	2.12	F89	HB3	3.14	R94	HA	3.86	I100	CG1	25.94
E84	HB3	2.26	F89	HD#	7.23	R94	CB	29.72	I100	HA	4.77
E84	HG2	2.35	F89	HE#	6.93	R94	CG	26.07	I100	HB	1.94
E84	HG3	2.45	F89	HZ	6.98	R94	HB2	1.72	I100	HG12	0.23
M85	HN	8.39	K90	HN	7.56	R94	HB3	1.86	I100	HG13	1.23
M85	N	120.60	K90	N	115.32	R94	HG2	1.61	I100	HD1#	0.35
M85	CA	58.10	K90	CA	58.18	R94	HG3	1.72	I100	HG2#	0.97
M85	CB	32.08	K90	HA	3.87	R94	CD	42.07	S101	HN	9.53
M85	CG	32.28	K90	CB	31.80	R94	HD2	2.98	S101	N	126.02
M85	CE	16.55	K90	HB#	1.93	R94	HD3	3.12	S101	CA	55.04
M85	HA	4.51	K90	HG2	1.52	D95	HA	4.55	S101	CB	62.68
M85	HB2	2.22	K90	HG3	1.76	D95	HN	8.24	S101	HA	5.10
M85	HB3	2.68	K90	CG	24.61	D95	N	114.06	S101	HB2	4.01
M85	HG2	2.60	K90	HD#	1.70	D95	CB	39.05	S101	HB3	4.43
M85	HG3	2.86	K90	CD	28.72	D95	CA	52.15	P102	N	107.50
M85	HE#	2.00	K90	CE	41.16	D95	HB2	2.59	P102	CA	65.64
R86	HN	8.60	K90	HE#	2.92	D95	HB3	3.07	P102	CB	30.61
R86	N	120.67	I91	HN	7.38	G96	HN	7.68	P102	CG	27.62
R86	CA	58.70	I91	N	117.99	G96	N	109.68	P102	CD	49.08
R86	HA	4.09	I91	CG2	16.05	G96	CA	46.57	P102	HA	4.11
R86	CB	28.91	I91	CA	62.79	G96	HA1	3.81	P102	HB2	2.02
R86	HB2	1.76	I91	CB	36.55	G96	HA2	3.88	P102	HB3	2.37
R86	HB3	2.05	I91	CG1	27.76	D97	HA	4.47	P102	HG2	2.10
R86	CG	26.23	I91	CD1	11.93	D97	HN	8.49	P102	HG3	2.35
R86	HG2	1.52	I91	HA	3.59	D97	N	121.08	P102	HD2	4.01
R86	HG3	1.76	I91	HB	2.02	D97	CB	39.58	P102	HD3	4.06
R86	CD	42.07	I91	HG12	1.21	D97	CA	53.23	A103	HN	8.01
R86	HD2	2.79	I91	HG13	1.66	D97	HB2	2.58	A103	N	117.32
R86	HD3	2.86	I91	HG2#	0.65	D97	HB3	3.16	A103	CA	54.29
E87	HN	8.09	I91	HD1#	0.83	G98	HN	10.41	A103	HA	4.10
E87	N	119.09	F92	HN	7.31	G98	N	112.41	A103	HB#	1.42
E87	CA	58.24	F92	N	117.09	G98	CA	44.18	A103	CB	17.79
E87	CB	28.40	F92	CA	59.06	G98	HA1	3.43	E104	HN	7.98
E87	CG	35.11	F92	CB	39.50	G98	HA2	4.02	E104	N	121.08
E87	HA	4.07	F92	HA	4.24	F99	HN	8.17	E104	CA	58.57
E87	HB2	2.13	F92	HB2	2.60	F99	N	117.13	E104	CB	28.45
E87	HB3	2.16	F92	HB3	2.64	F99	CA	55.29	E104	CG	37.06
E87	HG#	2.47	F92	HD#	7.30	F99	CB	43.62	E104	HA	4.07
A88	HN	8.14	F92	HE#	7.36	F99	HA	5.17	E104	HB2	2.63
A88	N	122.36	F92	HZ	6.99	F99	HB2	2.74	E104	HB3	2.67
A88	CA	54.33	D93	HA	4.50	F99	HB3	2.83	E104	HG#	2.24
A88	HA	4.21	D93	HN	7.81	F99	HD#	6.91	L105	HN	8.54

L105	N	120.65	M109	HB2	1.90	E114	HA	4.32	E119	HA	4.12
L105	CA	57.47	M109	HB3	2.18	E114	HB2	1.73	E119	HB2	1.96
L105	CB	41.30	M109	HG#	2.70	E114	HB3	1.96	E119	HB3	2.06
L105	CG	26.42	M109	HE#	2.02	E114	HG2	2.11	E119	HG2	2.32
L105	CD1	25.61	I110	HN	8.21	E114	HG3	2.25	E119	HG3	2.40
L105	CD2	23.28	I110	N	118.40	K115	HN	8.59	E120	HN	7.74
L105	HA	4.13	I110	CD1	12.13	K115	N	123.97	E120	N	120.67
L105	HB2	1.51	I110	CG2	16.20	K115	CA	54.84	E120	CA	58.29
L105	HB3	1.96	I110	CA	63.30	K115	HA	4.37	E120	CB	29.14
L105	HD1#	0.95	I110	CB	36.48	K115	CB	30.58	E120	CG	36.58
L105	HD2#	0.94	I110	CG1	28.28	K115	HB2	1.73	E120	HA	4.03
L105	HG	1.67	I110	HA	3.97	K115	HB3	1.78	E120	HB2	1.94
R106	HN	8.30	I110	HB	1.87	K115	HG2	1.33	E120	HB3	2.35
R106	N	117.09	I110	HG12	1.26	K115	HG3	1.42	E120	HG2	2.28
R106	CA	59.39	I110	HG13	1.63	K115	CG	23.68	E120	HG3	2.49
R106	HA	3.76	I110	HD1#	0.84	K115	CD	28.11	I121	HN	7.96
R106	CB	29.77	I110	HG2#	0.84	K115	CE	41.28	I121	N	121.65
R106	CG	26.30	N111	N	120.80	K115	HD2	1.63	I121	CD1	10.31
R106	CD	42.48	N111	HN	7.75	K115	HD3	1.66	I121	CG2	16.95
R106	HB#	1.90	N111	CA	55.40	K115	HE#	2.97	I121	CA	62.51
R106	HG2	1.69	N111	HA	4.38	V116	HN	7.74	I121	CB	35.35
R106	HG3	1.73	N111	CB	37.84	V116	N	120.09	I121	CG1	26.95
R106	HD2	3.18	N111	HB2	2.55	V116	CA	59.16	I121	HA	3.81
R106	HD3	3.24	N111	HB3	2.63	V116	CB	33.80	I121	HB	2.26
F107	HN	7.85	N111	ND2	112.90	V116	CG1	20.91	I121	HG12	1.45
F107	N	117.20	N111	HD21	6.19	V116	CG2	19.32	I121	HG13	1.49
F107	CA	60.67	N111	HD22	7.21	V116	HA	4.66	I121	HD1#	0.78
F107	CB	38.81	L112	HN	7.97	V116	HB	2.02	I121	HG2#	1.29
F107	HA	4.09	L112	N	118.80	V116	HG1#	0.89	D122	HA	4.33
F107	HB#	3.27	L112	CA	54.50	V116	HG2#	0.89	D122	HN	8.05
F107	HD#	7.20	L112	CB	41.52	T117	HN	8.54	D122	N	119.21
F107	HE#	7.23	L112	CG	25.50	T117	N	115.77	D122	CB	39.70
F107	HZ	7.13	L112	CD1	24.87	T117	CA	59.31	D122	CA	56.85
V108	HN	8.08	L112	CD2	21.59	T117	CB	70.81	D122	HB2	2.65
V108	N	118.70	L112	HA	4.31	T117	CG2	20.89	D122	HB3	2.78
V108	CA	65.65	L112	HB2	1.70	T117	HA	4.59	E123	HN	7.89
V108	CB	30.81	L112	HB3	1.91	T117	HB	4.72	E123	N	119.46
V108	CG1	22.51	L112	HD1#	0.81	T117	HG2#	1.29	E123	CA	58.46
V108	CG2	20.07	L112	HD2#	0.81	D118	HA	4.28	E123	CB	28.78
V108	HA	3.42	L112	HG	1.82	D118	HN	8.90	E123	CG	35.12
V108	HB	2.01	G113	HN	7.89	D118	N	121.26	E123	HA	4.01
V108	HG1#	0.93	G113	N	107.26	D118	CB	39.10	E123	HB2	2.09
V108	HG2#	0.32	G113	CA	44.64	D118	CA	57.16	E123	HB3	2.11
M109	CA	58.00	G113	HA1	4.17	D118	HB2	2.58	E123	HG2	2.27
M109	CB	30.97	G113	HA2	3.74	D118	HB3	2.75	E123	HG3	2.35
M109	HA	4.10	E114	HN	7.89	E119	HN	8.73	M124	HN	8.06
M109	HN	8.12	E114	N	119.87	E119	N	119.30	M124	N	119.75
M109	N	116.60	E114	CA	54.72	E119	CA	59.17	M124	CA	58.46
M109	CG	32.62	E114	CB	29.64	E119	CB	28.20	M124	CB	32.76
M109	CE	16.85	E114	CG	34.61	E119	CG	35.60	M124	CG	31.27

M124	CE	15.93	D129	CB	38.67	I136	N	125.02	F141	N	123.26
M124	HA	4.12	D129	CA	52.14	I136	CD1	10.45	F141	CA	60.48
M124	HB2	2.12	D129	HB2	2.43	I136	CG2	16.95	F141	CB	38.68
M124	HB3	2.35	D129	HB3	2.70	I136	CA	57.46	F141	HA	4.07
M124	HG2	2.68	F130	HN	8.22	I136	CB	37.19	F141	HB2	3.26
M124	HG3	2.77	F130	N	126.66	I136	CG1	25.89	F141	HB3	3.59
M124	HE#	2.08	F130	CA	58.66	I136	HA	5.40	F141	HD#	6.67
I125	HN	8.00	F130	CB	38.15	I136	HB	2.36	F141	HE#	6.93
I125	N	118.03	F130	HA	4.32	I136	HG1#	1.36	F141	HZ	7.13
I125	CD1	11.66	F130	HB2	3.02	I136	HD1#	0.82	V142	HN	8.39
I125	CG2	15.09	F130	HB3	3.18	I136	HG2#	1.29	V142	N	119.10
I125	CA	63.55	F130	HD#	7.08	N137	HN	9.40	V142	CA	65.59
I125	CB	36.23	F130	HE#	6.79	N137	N	129.16	V142	CB	30.81
I125	CG1	27.89	F130	HZ	7.13	N137	CA	50.33	V142	CG1	21.62
I125	HA	3.56	D131	HA	4.63	N137	CB	37.12	V142	CG2	20.20
I125	HB	2.04	D131	HN	8.19	N137	HA	5.22	V142	HA	3.23
I125	HG12	1.13	D131	N	114.88	N137	HB#	3.34	V142	HB	1.88
I125	HG13	1.69	D131	CB	39.00	N137	ND2	107.90	V142	HG1#	0.60
I125	HD1#	0.78	D131	CA	52.52	N137	HD21	6.71	V142	HG2#	0.77
I125	HG2#	0.71	D131	HB2	2.66	N137	HD22	7.24	W143	HN	7.62
R126	HN	8.12	D131	HB3	3.09	Y138	HN	8.41	W143	N	120.83
R126	N	117.48	G132	HN	7.51	Y138	N	118.02	W143	NE1	129.22
R126	CA	58.86	G132	N	108.56	Y138	CA	62.10	W143	CA	61.21
R126	HA	3.93	G132	CA	46.71	Y138	CB	36.99	W143	CB	27.87
R126	CB	29.34	G132	HA1	3.78	Y138	HA	3.55	W143	HA	4.03
R126	CG	26.90	G132	HA2	3.90	Y138	HB2	2.01	W143	HB2	3.35
R126	HB2	1.84	D133	HA	4.44	Y138	HB3	2.36	W143	HB3	3.73
R126	HB3	1.92	D133	HN	8.27	Y138	HD#	6.44	W143	HD1	7.26
R126	HG2	1.62	D133	N	120.79	Y138	HE#	6.52	W143	HE3	7.47
R126	HG3	1.75	D133	CB	39.25	E139	HN	8.02	W143	HZ2	7.45
R126	CD	42.45	D133	CA	52.81	E139	N	118.05	W143	HZ3	7.00
R126	HD#	3.20	D133	HB2	2.40	E139	CA	59.66	W143	HH2	7.13
E127	HN	7.90	D133	HB3	2.87	E139	CB	28.25	W143	HE1	10.10
E127	N	115.88	G134	HN	10.54	E139	CG	36.53	M144	HN	8.76
E127	CA	57.80	G134	N	113.72	E139	HA	3.67	M144	N	120.82
E127	CB	29.21	G134	CA	44.79	E139	HB2	2.00	M144	CA	58.84
E127	CG	35.75	G134	HA1	3.34	E139	HB3	2.13	M144	CB	33.29
E127	HA	3.91	G134	HA2	3.98	E139	HG2	2.31	M144	CG	30.61
E127	HB#	2.15	M135	HN	7.94	E139	HG3	2.39	M144	CE	16.05
E127	HG2	2.39	M135	N	115.78	E140	HN	8.62	M144	HA	3.72
E127	HG3	2.52	M135	CA	51.58	E140	N	121.51	M144	HB2	2.25
A128	HN	7.12	M135	CB	36.46	E140	CA	57.90	M144	HB3	2.31
A128	N	118.03	M135	CG	31.37	E140	CB	28.89	M144	HG2	2.25
A128	CA	50.86	M135	CE	17.25	E140	CG	36.37	M144	HG3	2.63
A128	HA	4.14	M135	HA	4.89	E140	HA	4.12	M144	HE#	1.90
A128	HB#	1.52	M135	HB2	1.67	E140	HB2	2.29	I145	HN	8.27
A128	CB	19.91	M135	HB3	1.80	E140	HB3	2.48	I145	N	115.32
D129	HA	4.55	M135	HG#	2.14	E140	HG2	2.47	I145	CD1	11.37
D129	HN	7.46	M135	HE#	1.76	E140	HG3	2.76	I145	CG2	17.13
D129	N	115.35	I136	HN	9.06	F141	HN	8.94	I145	CA	60.92

I145	CB	36.31	S146	CA	58.81	Q147	HB2	1.61	K148	HA	4.01
I145	CG1	25.86	S146	CB	62.73	Q147	HB3	1.90	K148	CB	32.42
I145	HA	3.84	S146	HA	4.30	Q147	HG2	1.52	K148	HB2	1.60
I145	HB	1.54	S146	HB#	3.81	Q147	HG3	1.77	K148	HB3	1.74
I145	HG12	0.79	Q147	HN	7.25	Q147	NE2	113.80	K148	HG#	1.31
I145	HG13	0.86	Q147	N	119.65	Q147	HE21	6.26	K148	CG	23.72
I145	HD1#	0.46	Q147	CA	54.45	Q147	HE22	6.65	K148	HD#	1.55
I145	HG2#	0.48	Q147	CB	27.76	K148	HN	7.49	K148	CD	28.05
S146	HN	7.54	Q147	CG	31.73	K148	N	126.54	K148	CE	41.18
S146	N	116.16	Q147	HA	3.95	K148	CA	56.80	K148	HE#	2.85

B.2 Hydrogen bonds restraints used in structure calculations holo androcam

File Name : "hbonds-Ca.tbl"

```
assign (residue 6 and name O) (residue 10 and name HN)  1.80  0.00 0.50
assign ( residue   6 and name O  ) ( residue   10  and name N  )  2.80  0.00  0.50
assign ( residue   7 and name O  ) ( residue   11  and name HN )  1.80  0.00  0.50
assign ( residue   7 and name O  ) ( residue   11  and name N  )  2.80  0.00  0.50
assign ( residue   8 and name O  ) ( residue   12  and name HN )  1.80  0.00  0.50
assign ( residue   8 and name O  ) ( residue   12  and name N  )  2.80  0.00  0.50
assign ( residue   9 and name O  ) ( residue   13  and name HN )  1.80  0.00  0.50
assign ( residue   9 and name O  ) ( residue   13  and name N  )  2.80  0.00  0.50
assign ( residue   10 and name O  ) ( residue   14  and name HN )  1.80  0.00  0.50
assign ( residue   10 and name O  ) ( residue   14  and name N  )  2.80  0.00  0.50
assign ( residue   11 and name O  ) ( residue   15  and name HN )  1.80  0.00  0.50
assign ( residue   11 and name O  ) ( residue   15  and name N  )  2.80  0.00  0.50
assign ( residue   12 and name O  ) ( residue   16  and name HN )  1.80  0.00  0.50
assign ( residue   12 and name O  ) ( residue   16  and name N  )  2.80  0.00  0.50
assign ( residue   13 and name O  ) ( residue   17  and name HN )  1.80  0.00  0.50
assign ( residue   13 and name O  ) ( residue   17  and name N  )  2.80  0.00  0.50
assign ( residue   14 and name O  ) ( residue   18  and name HN )  1.80  0.00  0.50
assign ( residue   14 and name O  ) ( residue   18  and name N  )  2.80  0.00  0.50
assign ( residue   32 and name O  ) ( residue   36  and name HN )  1.80  0.00  0.50
assign ( residue   32 and name O  ) ( residue   36  and name N  )  2.80  0.00  0.50
assign ( residue   33 and name O  ) ( residue   37  and name HN )  1.80  0.00  0.50
assign ( residue   33 and name O  ) ( residue   37  and name N  )  2.80  0.00  0.50
assign ( residue   34 and name O  ) ( residue   38  and name HN )  1.80  0.00  0.50
assign ( residue   34 and name O  ) ( residue   38  and name N  )  2.80  0.00  0.50
assign ( residue   45 and name O  ) ( residue   49  and name HN )  1.80  0.00  0.50
assign ( residue   45 and name O  ) ( residue   49  and name N  )  2.80  0.00  0.50
assign ( residue   46 and name O  ) ( residue   50  and name HN )  1.80  0.00  0.50
assign ( residue   46 and name O  ) ( residue   50  and name N  )  2.80  0.00  0.50
assign ( residue   47 and name O  ) ( residue   51  and name HN )  1.80  0.00  0.50
assign ( residue   47 and name O  ) ( residue   51  and name N  )  2.80  0.00  0.50
assign ( residue   48 and name O  ) ( residue   52  and name HN )  1.80  0.00  0.50
assign ( residue   48 and name O  ) ( residue   52  and name N  )  2.80  0.00  0.50
assign ( residue   49 and name O  ) ( residue   53  and name HN )  1.80  0.00  0.50
assign ( residue   49 and name O  ) ( residue   53  and name N  )  2.80  0.00  0.50
assign ( residue   50 and name O  ) ( residue   54  and name HN )  1.80  0.00  0.50
assign ( residue   50 and name O  ) ( residue   54  and name N  )  2.80  0.00  0.50
assign ( residue   51 and name O  ) ( residue   55  and name HN )  1.80  0.00  0.50
assign ( residue   51 and name O  ) ( residue   55  and name N  )  2.80  0.00  0.50
assign ( residue   52 and name O  ) ( residue   56  and name HN )  1.80  0.00  0.50
assign ( residue   52 and name O  ) ( residue   56  and name N  )  2.80  0.00  0.50
assign ( residue   65 and name O  ) ( residue   69  and name HN )  1.80  0.00  0.50
assign ( residue   65 and name O  ) ( residue   69  and name N  )  2.80  0.00  0.50
assign ( residue   66 and name O  ) ( residue   70  and name HN )  1.80  0.00  0.50
assign ( residue   66 and name O  ) ( residue   70  and name N  )  2.80  0.00  0.50
assign ( residue   67 and name O  ) ( residue   71  and name HN )  1.80  0.00  0.50
assign ( residue   67 and name O  ) ( residue   71  and name N  )  2.80  0.00  0.50
assign ( residue   68 and name O  ) ( residue   72  and name HN )  1.80  0.00  0.50
assign ( residue   68 and name O  ) ( residue   72  and name N  )  2.80  0.00  0.50
assign ( residue   69 and name O  ) ( residue   73  and name HN )  1.80  0.00  0.50
assign ( residue   69 and name O  ) ( residue   73  and name N  )  2.80  0.00  0.50
assign ( residue   70 and name O  ) ( residue   74  and name HN )  1.80  0.00  0.50
assign ( residue   70 and name O  ) ( residue   74  and name N  )  2.80  0.00  0.50
assign ( residue   71 and name O  ) ( residue   75  and name HN )  1.80  0.00  0.50
assign ( residue   71 and name O  ) ( residue   75  and name N  )  2.80  0.00  0.50
assign ( residue   72 and name O  ) ( residue   76  and name HN )  1.80  0.00  0.50
assign ( residue   72 and name O  ) ( residue   76  and name N  )  2.80  0.00  0.50
assign ( residue   73 and name O  ) ( residue   77  and name HN )  1.80  0.00  0.50
assign ( residue   73 and name O  ) ( residue   77  and name N  )  2.80  0.00  0.50
assign ( residue   82 and name O  ) ( residue   86  and name HN )  1.80  0.00  0.50
assign ( residue   82 and name O  ) ( residue   86  and name N  )  2.80  0.00  0.50
assign ( residue   83 and name O  ) ( residue   87  and name HN )  1.80  0.00  0.50
assign ( residue   83 and name O  ) ( residue   87  and name N  )  2.80  0.00  0.50
assign ( residue   84 and name O  ) ( residue   88  and name HN )  1.80  0.00  0.50
assign ( residue   84 and name O  ) ( residue   88  and name N  )  2.80  0.00  0.50
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assign ( residue   85 and name O  ) ( residue   89  and name HN )  1.80  0.00  0.50
assign ( residue   85 and name O  ) ( residue   89  and name N  )  2.80  0.00  0.50
assign ( residue   86 and name O  ) ( residue   90  and name HN )  1.80  0.00  0.50
assign ( residue   86 and name O  ) ( residue   90  and name N  )  2.80  0.00  0.50
assign ( residue   87 and name O  ) ( residue   91  and name HN )  1.80  0.00  0.50
assign ( residue   87 and name O  ) ( residue   91  and name N  )  2.80  0.00  0.50
assign ( residue   88 and name O  ) ( residue   92  and name HN )  1.80  0.00  0.50
assign ( residue   88 and name O  ) ( residue   92  and name N  )  2.80  0.00  0.50
assign ( residue   102 and name O  ) ( residue   106  and name HN )  1.80  0.00  0.50
assign ( residue   102 and name O  ) ( residue   106  and name N  )  2.80  0.00  0.50
assign ( residue   103 and name O  ) ( residue   107  and name HN )  1.80  0.00  0.50
assign ( residue   103 and name O  ) ( residue   107  and name N  )  2.80  0.00  0.50
assign ( residue   104 and name O  ) ( residue   108  and name HN )  1.80  0.00  0.50
assign ( residue   104 and name O  ) ( residue   108  and name N  )  2.80  0.00  0.50
assign ( residue   105 and name O  ) ( residue   109  and name HN )  1.80  0.00  0.50
assign ( residue   105 and name O  ) ( residue   109  and name N  )  2.80  0.00  0.50
assign ( residue   118 and name O  ) ( residue   122  and name HN )  1.80  0.00  0.50
assign ( residue   118 and name O  ) ( residue   122  and name N  )  2.80  0.00  0.50
assign ( residue   119 and name O  ) ( residue   123  and name HN )  1.80  0.00  0.50
assign ( residue   119 and name O  ) ( residue   123  and name N  )  2.80  0.00  0.50
assign ( residue   120 and name O  ) ( residue   124  and name HN )  1.80  0.00  0.50
assign ( residue   120 and name O  ) ( residue   124  and name N  )  2.80  0.00  0.50
assign ( residue   121 and name O  ) ( residue   125  and name HN )  1.80  0.00  0.50
assign ( residue   121 and name O  ) ( residue   125  and name N  )  2.80  0.00  0.50
assign ( residue   122 and name O  ) ( residue   126  and name HN )  1.80  0.00  0.50
assign ( residue   122 and name O  ) ( residue   126  and name N  )  2.80  0.00  0.50
assign ( residue   123 and name O  ) ( residue   127  and name HN )  1.80  0.00  0.50
assign ( residue   123 and name O  ) ( residue   127  and name N  )  2.80  0.00  0.50
assign ( residue   138 and name O  ) ( residue   142  and name HN )  1.80  0.00  0.50
assign ( residue   138 and name O  ) ( residue   142  and name N  )  2.80  0.00  0.50
assign ( residue   139 and name O  ) ( residue   143  and name HN )  1.80  0.00  0.50
assign ( residue   139 and name O  ) ( residue   143  and name N  )  2.80  0.00  0.50
assign ( residue   140 and name O  ) ( residue   144  and name HN )  1.80  0.00  0.50
assign ( residue   140 and name O  ) ( residue   144  and name N  )  2.80  0.00  0.50
assign ( residue   141 and name O  ) ( residue   145  and name HN )  1.80  0.00  0.50
assign ( residue   141 and name O  ) ( residue   145  and name N  )  2.80  0.00  0.50
assign ( residue   142 and name O  ) ( residue   146  and name HN )  1.80  0.00  0.50
assign ( residue   142 and name O  ) ( residue   146  and name N  )  2.80  0.00  0.50

! BETA sheet hydrogen bonding in N lobe

assign ( residue   27 and name O  ) ( residue   63  and name HN )  1.80  0.00  0.40
assign ( residue   27 and name O  ) ( residue   63  and name N  )  2.80  0.00  0.40
assign ( residue   63 and name O  ) ( residue   27  and name HN )  1.80  0.00  0.40
assign ( residue   63 and name O  ) ( residue   27  and name N  )  2.80  0.00  0.40

! BETA sheet hydrogen bonding in C lobe

assign ( residue   100 and name O  ) ( residue   136  and name HN )  1.80  0.00  0.40
assign ( residue   100 and name O  ) ( residue   136  and name N  )  2.80  0.00  0.40

assign ( residue   136 and name O  ) ( residue   100  and name HN )  1.80  0.00  0.40
assign ( residue   136 and name O  ) ( residue   100  and name N  )  2.80  0.00  0.40

! Ca+2 tying in the C lobe

assign ( residue   149  ) ( residue   93  and name OD1 )  2.27  0.00  0.00
assign ( residue   149  ) ( residue   95  and name OD1  )  2.45  0.00  0.00
assign ( residue   149  ) ( residue   97  and name OD1  )  2.36  0.00  0.00
assign ( residue   149  ) ( residue   99  and name O  )  2.29  0.00  0.00
assign ( residue   149  ) ( residue   104  and name OE1  )  2.46  0.00  0.00
assign ( residue   149  ) ( residue   104  and name OE2  )  2.52  0.00  0.00
assign ( residue   150  ) ( residue   129  and name OD1 )  2.27  0.00  0.00
assign ( residue   150  ) ( residue   131  and name OD1  )  2.45  0.00  0.00
assign ( residue   150  ) ( residue   133  and name OD1  )  2.36  0.00  0.00
assign ( residue   150  ) ( residue   135  and name O  )  2.29  0.00  0.00
assign ( residue   150  ) ( residue   140  and name OE1  )  2.46  0.00  0.00
assign ( residue   150  ) ( residue   140  and name OE2  )  2.52  0.00  0.00
```

B.3 χ_1, φ, ψ dihedral restraints for holo androcam

File name : "di.tbl"

```
!   ACaM backbone "phi" angles derived on 2008/07/24  from HNHA and CSI data
!  E6
assign (resid 5 and name C)  (resid 6 and name N) (resid 6 and name CA) (resid 6 and name
C)  1.00  -60.00  20.00  2
!  E7
assign (resid 6 and name C) (resid 7 and name N) (resid 7 and name CA) (resid 7 and name
C)  1.00  -60.00  20.00  2
!  Q8
assign (resid 7 and name C) (resid 8 and name N) (resid 8 and name CA) (resid 8 and name
C)  1.00  -60.00  20.00  2
!  I9
assign (resid 8 and name C) (resid 9 and name N) (resid 9 and name CA) (resid 9 and name
C)  1.00  -60.00  20.00  2
!  A10
assign (resid 9 and name C) (resid 10 and name N) (resid 10 and name CA) (resid 10 and
name C)  1.00  -60.00  20.00  2
!  E11
assign (resid 10 and name C) (resid 11 and name N) (resid 11 and name CA) (resid 11 and
name C)  1.00  -60.00  20.00  2
!  F12
assign (resid 11 and name C) (resid 12 and name N) (resid 12 and name CA) (resid 12 and
name C)  1.00  -60.00  20.00  2
!  K13
assign (resid 12 and name C) (resid 13 and name N) (resid 13 and name CA) (resid 13 and
name C)  1.00  -60.00  20.00  2
!  D14
assign (resid 13 and name C) (resid 14 and name N) (resid 14 and name CA) (resid 14 and
name C)  1.00  -60.00  20.00  2
!  A15
assign (resid 14 and name C) (resid 15 and name N) (resid 15 and name CA) (resid 15 and
name C)  1.00  -60.00  20.00  2
!  F16
assign (resid 15 and name C) (resid 16 and name N) (resid 16 and name CA) (resid 16 and
name C)  1.00  -60.00  20.00  2
!  V17
assign (resid 16 and name C) (resid 17 and name N) (resid 17 and name CA) (resid 17 and
name C)  1.00  -60.00  20.00  2
!  Q18
assign (resid 17 and name C) (resid 18 and name N) (resid 18 and name CA) (resid 18 and
name C)  1.00  -60.00  20.00  2
!  K21
assign (resid 20 and name C) (resid 21 and name N) (resid 21 and name CA) (resid 21 and
name C)  1.00  -60.00  40.00  2
!  K26
assign (resid 25 and name C) (resid 26 and name N) (resid 26 and name CA) (resid 26 and
name C)  1.00  -135.00  20.00  2
!  I27
assign (resid 26 and name C) (resid 27 and name N) (resid 27 and name CA) (resid 27 and
name C)  1.00  -135.00  20.00  2
!  T29
assign (resid 28 and name C) (resid 29 and name N) (resid 29 and name CA) (resid 29 and
name C)  1.00  -60.00  40.00  2


!  R30
assign (resid 29 and name C) (resid 30 and name N) (resid 30 and name CA) (resid 30 and
name C)  1.00  -60.00  40.00  2
!  L32
assign (resid 31 and name C) (resid 32 and name N) (resid 32 and name CA) (resid 32 and
name C)  1.00  -60.00  20.00  2
!  G33
assign (resid 32 and name C) (resid 33 and name N) (resid 33 and name CA) (resid 33 and
name C)  1.00  -60.00  20.00  2
!  T34
```

```
assign (resid 33 and name C) (resid 34 and name N) (resid 34 and name CA) (resid 34 and
name C)  1.00  -60.00  20.00  2
!  L35
assign (resid 34 and name C) (resid 35 and name N) (resid 35 and name CA) (resid 35 and
name C)  1.00  -60.00  20.00  2
!  M36
assign (resid 35 and name C) (resid 36 and name N) (resid 36 and name CA) (resid 36 and
name C)  1.00  -60.00  20.00  2
!  R37
assign (resid 36 and name C) (resid 37 and name N) (resid 37 and name CA) (resid 37 and
name C)  1.00  -60.00  20.00  2
!  T38
assign (resid 37 and name C) (resid 38 and name N) (resid 38 and name CA) (resid 38 and
name C)  1.00  -60.00  20.00  2
!  L39
assign (resid 38 and name C) (resid 39 and name N) (resid 39 and name CA) (resid 39 and
name C)  1.00  -135.00  20.00  2
!  E45
assign (resid 44 and name C) (resid 45 and name N) (resid 45 and name CA) (resid 45 and
name C)  1.00  -60.00  20.00  2
!  A46
assign (resid 45 and name C) (resid 46 and name N) (resid 46 and name CA) (resid 46 and
name C)  1.00  -60.00  20.00  2
!  E47
assign (resid 46 and name C) (resid 47 and name N) (resid 47 and name CA) (resid 47 and
name C)  1.00  -60.00  20.00  2
!  L48
assign (resid 47 and name C) (resid 48 and name N) (resid 48 and name CA) (resid 48 and
name C)  1.00  -60.00  20.00  2
!  Q49
assign (resid 48 and name C) (resid 49 and name N) (resid 49 and name CA) (resid 49 and
name C)  1.00  -60.00  20.00  2
!  D50
assign (resid 49 and name C) (resid 50 and name N) (resid 50 and name CA) (resid 50 and
name C)  1.00  -60.00  20.00  2
!  L51
assign (resid 50 and name C) (resid 51 and name N) (resid 51 and name CA) (resid 51 and
name C)  1.00  -60.00  20.00  2
!  I52
assign (resid 51 and name C) (resid 52 and name N) (resid 52 and name CA) (resid 52 and
name C)  1.00  -60.00  20.00  2
!  A53
assign (resid 52 and name C) (resid 53 and name N) (resid 53 and name CA) (resid 53 and
name C)  1.00  -60.00  20.00  2
!  E54
assign (resid 53 and name C) (resid 54 and name N) (resid 54 and name CA) (resid 54 and
name C)  1.00  -60.00  20.00  2
!  A55
assign (resid 54 and name C) (resid 55 and name N) (resid 55 and name CA) (resid 55 and
name C)  1.00  -60.00  20.00  2
!  E56
assign (resid 55 and name C) (resid 56 and name N) (resid 56 and name CA) (resid 56 and
name C)  1.00  -60.00  20.00  2
!  Q62
assign (resid 61 and name C) (resid 62 and name N) (resid 62 and name CA) (resid 62 and
name C)  1.00  -135.00  20.00  2
!  L63
assign (resid 62 and name C) (resid 63 and name N) (resid 63 and name CA) (resid 63 and
name C)  1.00  -135.00  20.00  2
!  N64
assign (resid 63 and name C) (resid 64 and name N) (resid 64 and name CA) (resid 64 and
name C)  1.00  -135.00  20.00  2
!
assign (resid 64 and name C) (resid 65 and name N) (resid 65 and name CA) (resid 65 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 65 and name C) (resid 66 and name N) (resid 66 and name CA) (resid 66 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 66 and name C) (resid 67 and name N) (resid 67 and name CA) (resid 67 and
name C)  1.00  -60.00  20.00  2
```

```
!
assign (resid 67 and name C) (resid 68 and name N) (resid 68 and name CA) (resid 68 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 68 and name C) (resid 69 and name N) (resid 69 and name CA) (resid 69 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 69 and name C) (resid 70 and name N) (resid 70 and name CA) (resid 70 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 70 and name C) (resid 71 and name N) (resid 71 and name CA) (resid 71 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 71 and name C) (resid 72 and name N) (resid 72 and name CA) (resid 72 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 72 and name C) (resid 73 and name N) (resid 73 and name CA) (resid 73 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 73 and name C) (resid 74 and name N) (resid 74 and name CA) (resid 74 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 74 and name C) (resid 75 and name N) (resid 75 and name CA) (resid 75 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 75 and name C) (resid 76 and name N) (resid 76 and name CA) (resid 76 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 76 and name C) (resid 77 and name N) (resid 77 and name CA) (resid 77 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 81 and name C) (resid 82 and name N) (resid 82 and name CA) (resid 82 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 82 and name C) (resid 83 and name N) (resid 83 and name CA) (resid 83 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 83 and name C) (resid 84 and name N) (resid 84 and name CA) (resid 84 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 84 and name C) (resid 85 and name N) (resid 85 and name CA) (resid 85 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 85 and name C) (resid 86 and name N) (resid 86 and name CA) (resid 86 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 86 and name C) (resid 87 and name N) (resid 87 and name CA) (resid 87 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 87 and name C) (resid 88 and name N) (resid 88 and name CA) (resid 88 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 88 and name C) (resid 89 and name N) (resid 89 and name CA) (resid 89 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 89 and name C) (resid 90 and name N) (resid 90 and name CA) (resid 90 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 90 and name C) (resid 91 and name N) (resid 91 and name CA) (resid 91 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 91 and name C) (resid 92 and name N) (resid 92 and name CA) (resid 92 and
name C)  1.00  -60.00  20.00  2
!
assign (resid 93 and name C) (resid 94 and name N) (resid 94 and name CA) (resid 94 and
name C)  1.00  -60.00  40.00  2
!
assign (resid 95 and name C) (resid 96 and name N) (resid 96 and name CA) (resid 96 and
name C)  1.00  -60.00  40.00  2
!
```

```
assign (resid 98 and name C) (resid 99 and name N) (resid 99 and name CA) (resid 99 and
name C)  1.00  -135.00  20.00  2
!
assign (resid 99 and name C) (resid 100 and name N) (resid 100 and name CA) (resid 100
and name C)  1.00  -135.00  20.00  2
!
assign (resid 100 and name C) (resid 101 and name N) (resid 101 and name CA) (resid 101
and name C)  1.00  -135.00  20.00  2
!
assign (resid 101 and name C) (resid 102 and name N) (resid 102 and name CA) (resid 102
and name C)  1.00  -60.00  20.00  2
!
assign (resid 102 and name C) (resid 103 and name N) (resid 103 and name CA) (resid 103
and name C)  1.00  -60.00  20.00  2
!
assign (resid 103 and name C) (resid 104 and name N) (resid 104 and name CA) (resid 104
and name C)  1.00  -60.00  20.00  2
!
assign (resid 104 and name C) (resid 105 and name N) (resid 105 and name CA) (resid 105
and name C)  1.00  -60.00  20.00  2
!
assign (resid 105 and name C) (resid 106 and name N) (resid 106 and name CA) (resid 106
and name C)  1.00  -60.00  20.00  2
!
assign (resid 106 and name C) (resid 107 and name N) (resid 107 and name CA) (resid 107
and name C)  1.00  -60.00  20.00  2
!
assign (resid 107 and name C) (resid 108 and name N) (resid 108 and name CA) (resid 108
and name C)  1.00  -60.00  20.00  2
!
assign (resid 108 and name C) (resid 109 and name N) (resid 109 and name CA) (resid 109
and name C)  1.00  -60.00  20.00  2
!
assign (resid 110 and name C) (resid 111 and name N) (resid 111 and name CA) (resid 111
and name C)  1.00  -60.00  20.00  2
!
assign (resid 115 and name C) (resid 116 and name N) (resid 116 and name CA) (resid 116
and name C)  1.00  -135.00  20.00  2
!
assign (resid 117 and name C) (resid 118 and name N) (resid 118 and name CA) (resid 118
and name C)  1.00  -60.00  20.00  2
!
assign (resid 118 and name C) (resid 119 and name N) (resid 119 and name CA) (resid 119
and name C)  1.00  -60.00  20.00  2
!
assign (resid 119 and name C) (resid 120 and name N) (resid 120 and name CA) (resid 120
and name C)  1.00  -60.00  20.00  2
!
assign (resid 120 and name C) (resid 121 and name N) (resid 121 and name CA) (resid 121
and name C)  1.00  -60.00  20.00  2
!
assign (resid 121 and name C) (resid 122 and name N) (resid 122 and name CA) (resid 122
and name C)  1.00  -60.00  20.00  2
!
assign (resid 122 and name C) (resid 123 and name N) (resid 123 and name CA) (resid 123
and name C)  1.00  -60.00  20.00  2
!
assign (resid 123 and name C) (resid 124 and name N) (resid 124 and name CA) (resid 124
and name C)  1.00  -60.00  20.00  2
!
assign (resid 124 and name C) (resid 125 and name N) (resid 125 and name CA) (resid 125
and name C)  1.00  -60.00  20.00  2
!
assign (resid 125 and name C) (resid 126 and name N) (resid 126 and name CA) (resid 126
and name C)  1.00  -60.00  20.00  2
!
assign (resid 126 and name C) (resid 127 and name N) (resid 127 and name CA) (resid 127
and name C)  1.00  -60.00  20.00  2
!
assign (resid 129 and name C) (resid 130 and name N) (resid 130 and name CA) (resid 130
and name C)  1.00  -60.00  40.00  2
```

```
!
assign (resid 134 and name C) (resid 135 and name N) (resid 135 and name CA) (resid 135
and name C)  1.00  -135.00  20.00  2
!
assign (resid 135 and name C) (resid 136 and name N) (resid 136 and name CA) (resid 136
and name C)  1.00  -135.00  20.00  2
!
assign (resid 136 and name C) (resid 137 and name N) (resid 137 and name CA) (resid 137
and name C)  1.00  -135.00  20.00  2
!
assign (resid 137 and name C) (resid 138 and name N) (resid 138 and name CA) (resid 138
and name C)  1.00  -60.00  20.00  2
!
assign (resid 138 and name C) (resid 139 and name N) (resid 139 and name CA) (resid 139
and name C)  1.00  -60.00  20.00  2
!
assign (resid 139 and name C) (resid 140 and name N) (resid 140 and name CA) (resid 140
and name C)  1.00  -60.00  20.00  2
!
assign (resid 140 and name C) (resid 141 and name N) (resid 141 and name CA) (resid 141
and name C)  1.00  -60.00  20.00  2
!
assign (resid 141 and name C) (resid 142 and name N) (resid 142 and name CA) (resid 142
and name C)  1.00  -60.00  20.00  2
!
assign (resid 142 and name C) (resid 143 and name N) (resid 143 and name CA) (resid 143
and name C)  1.00  -60.00  20.00  2
!
assign (resid 143 and name C) (resid 144 and name N) (resid 144 and name CA) (resid 144
and name C)  1.00  -60.00  20.00  2
!
assign (resid 144 and name C) (resid 145 and name N) (resid 145 and name CA) (resid 145
and name C)  1.00  -60.00  20.00  2
!
assign (resid 145 and name C) (resid 146 and name N) (resid 146 and name CA) (resid 146
and name C)  1.00  -60.00  20.00  2

!   ACaM backbone "psi" angles derived on 2008/07/24  from HNHA and CSI data

!  E6
assign (resid   6 and name N ) (resid   6 and name CA )  (resid   6 and name C ) (resid
7 and name N )  1.00  -35.00   20.00   2
!  E7
assign (resid   7 and name N ) (resid   7 and name CA )  (resid   7 and name C ) (resid
8 and name N )  1.00  -35.00   20.00   2
!  Q8
assign (resid   8 and name N ) (resid   8 and name CA )  (resid   8 and name C ) (resid
9 and name N )  1.00  -35.00   20.00   2
!  I9
assign (resid   9 and name N ) (resid   9 and name CA )  (resid   9 and name C ) (resid
10 and name N )  1.00  -35.00   20.00   2
!  A10
assign (resid   10 and name N ) (resid   10 and name CA )  (resid   10 and name C )
(resid   11 and name N )  1.00  -35.00   20.00   2
!  E11
assign (resid   11 and name N ) (resid   11 and name CA )  (resid   11 and name C )
(resid   12 and name N )  1.00  -35.00   20.00   2
!  F12
assign (resid   12 and name N ) (resid   12 and name CA )  (resid   12 and name C )
(resid   13 and name N )  1.00  -35.00   20.00   2


!  K13
assign (resid   13 and name N ) (resid   13 and name CA )  (resid   13 and name C )
(resid   14 and name N )  1.00  -35.00   20.00   2
!  D14
assign (resid   14 and name N ) (resid   14 and name CA )  (resid   14 and name C )
(resid   15 and name N )  1.00  -35.00   20.00   2
!  A15
assign (resid   15 and name N ) (resid   15 and name CA )  (resid   15 and name C )
(resid   16 and name N )  1.00  -35.00   20.00   2
```

```
!  F16
assign (resid   16 and name N ) (resid   16 and name CA )  (resid   16 and name C )
(resid   17 and name N )  1.00  -35.00   20.00   2
!  V17
assign (resid   17 and name N ) (resid   17 and name CA )  (resid   17 and name C )
(resid   18 and name N )  1.00  -35.00   20.00   2
!  Q18
assign (resid   18 and name N ) (resid   18 and name CA )  (resid   18 and name C )
(resid   19 and name N )  1.00  -35.00   20.00   2
!  K21
assign (resid   21 and name N ) (resid   21 and name CA )  (resid   21 and name C )
(resid   22 and name N )  1.00  -35.00   40.00   2
!  K26
assign (resid   26 and name N ) (resid   26 and name CA )  (resid   26 and name C )
(resid   27 and name N )  1.00  135.00   20.00   2
!  I27
assign (resid   27 and name N ) (resid   27 and name CA )  (resid   27 and name C )
(resid   28 and name N )  1.00  135.00   20.00   2
!  T29
assign (resid   29 and name N ) (resid   29 and name CA )  (resid   29 and name C )
(resid   30 and name N )  1.00  -35.00   40.00   2
!  R30
assign (resid   30 and name N ) (resid   30 and name CA )  (resid   30 and name C )
(resid   31 and name N )  1.00  -35.00   40.00   2
!  L32
assign (resid   32 and name N ) (resid   32 and name CA )  (resid   32 and name C )
(resid   33 and name N )  1.00  -35.00   20.00   2
!  G33
assign (resid   33 and name N ) (resid   33 and name CA )  (resid   33 and name C )
(resid   34 and name N )  1.00  -35.00   20.00   2
!  T34
assign (resid   34 and name N ) (resid   34 and name CA )  (resid   34 and name C )
(resid   35 and name N )  1.00  -35.00   20.00   2
!  L35
assign (resid   35 and name N ) (resid   35 and name CA )  (resid   35 and name C )
(resid   36 and name N )  1.00  -35.00   20.00   2
!  M36
assign (resid   36 and name N ) (resid   36 and name CA )  (resid   36 and name C )
(resid   37 and name N )  1.00  -35.00   20.00   2
!  R37
assign (resid   37 and name N ) (resid   37 and name CA )  (resid   37 and name C )
(resid   38 and name N )  1.00  -35.00   20.00   2
!  T38
assign (resid   38 and name N ) (resid   38 and name CA )  (resid   38 and name C )
(resid   39 and name N )  1.00  -35.00   20.00   2
!  L39
assign (resid   39 and name N ) (resid   39 and name CA )  (resid   39 and name C )
(resid   40 and name N )  1.00  135.00   20.00   2
!  E45
assign (resid   45 and name N ) (resid   45 and name CA )  (resid   45 and name C )
(resid   46 and name N )  1.00  -35.00   20.00   2
!  A46
assign (resid   46 and name N ) (resid   46 and name CA )  (resid   46 and name C )
(resid   47 and name N )  1.00  -35.00   20.00   2
!  E47
assign (resid   47 and name N ) (resid   47 and name CA )  (resid   47 and name C )
(resid   48 and name N )  1.00  -35.00   20.00   2
!  L48
assign (resid   48 and name N ) (resid   48 and name CA )  (resid   48 and name C )
(resid   49 and name N )  1.00  -35.00   20.00   2
!  Q49
assign (resid   49 and name N ) (resid   49 and name CA )  (resid   49 and name C )
(resid   50 and name N )  1.00  -35.00   20.00   2
!  D50
assign (resid   50 and name N ) (resid   50 and name CA )  (resid   50 and name C )
(resid   51 and name N )  1.00  -35.00   20.00   2
!  L51
assign (resid   51 and name N ) (resid   51 and name CA )  (resid   51 and name C )
(resid   52 and name N )  1.00  -35.00   20.00   2
!  I52
```

```
assign (resid   52 and name N ) (resid   52 and name CA )  (resid   52 and name C )
(resid   53 and name N )  1.00  -35.00   20.00   2
!  A53
assign (resid   53 and name N ) (resid   53 and name CA )  (resid   53 and name C )
(resid   54 and name N )  1.00  -35.00   20.00   2
!  E54
assign (resid   54 and name N ) (resid   54 and name CA )  (resid   54 and name C )
(resid   55 and name N )  1.00  -35.00   20.00   2
!  A55
assign (resid   55 and name N ) (resid   55 and name CA )  (resid   55 and name C )
(resid   56 and name N )  1.00  -35.00   20.00   2
!  E56
assign (resid   56 and name N ) (resid   56 and name CA )  (resid   56 and name C )
(resid   57 and name N )  1.00  -35.00   20.00   2
!  Q62
assign (resid   62 and name N ) (resid   62 and name CA )  (resid   62 and name C )
(resid   63 and name N )  1.00  135.00   20.00   2
!  L63
assign (resid   63 and name N ) (resid   63 and name CA )  (resid   63 and name C )
(resid   64 and name N )  1.00  135.00   20.00   2
!  N64
assign (resid   64 and name N ) (resid   64 and name CA )  (resid   64 and name C )
(resid   65 and name N )  1.00  135.00   20.00   2
!
assign (resid   65 and name N ) (resid   65 and name CA )  (resid   65 and name C )
(resid   66 and name N )  1.00  -35.00   20.00   2
!
assign (resid   66 and name N ) (resid   66 and name CA )  (resid   66 and name C )
(resid   67 and name N )  1.00  -35.00   20.00   2
!
assign (resid   67 and name N ) (resid   67 and name CA )  (resid   67 and name C )
(resid   68 and name N )  1.00  -35.00   20.00   2
!
assign (resid   68 and name N ) (resid   68 and name CA )  (resid   68 and name C )
(resid   69 and name N )  1.00  -35.00   20.00   2
!
assign (resid   69 and name N ) (resid   69 and name CA )  (resid   69 and name C )
(resid   70 and name N )  1.00  -35.00   20.00   2
!
assign (resid   70 and name N ) (resid   70 and name CA )  (resid   70 and name C )
(resid   71 and name N )  1.00  -35.00   20.00   2
!
assign (resid   71 and name N ) (resid   71 and name CA )  (resid   71 and name C )
(resid   72 and name N )  1.00  -35.00   20.00   2
!
assign (resid   72 and name N ) (resid   72 and name CA )  (resid   72 and name C )
(resid   73 and name N )  1.00  -35.00   20.00   2
!
assign (resid   73 and name N ) (resid   73 and name CA )  (resid   73 and name C )
(resid   74 and name N )  1.00  -35.00   20.00   2
!
assign (resid   74 and name N ) (resid   74 and name CA )  (resid   74 and name C )
(resid   75 and name N )  1.00  -35.00   20.00   2
!
assign (resid   75 and name N ) (resid   75 and name CA )  (resid   75 and name C )
(resid   76 and name N )  1.00  -35.00   20.00   2
!
assign (resid   76 and name N ) (resid   76 and name CA )  (resid   76 and name C )
(resid   77 and name N )  1.00  -35.00   20.00   2
!
assign (resid   77 and name N ) (resid   77 and name CA )  (resid   77 and name C )
(resid   78 and name N )  1.00  -35.00   20.00   2
!
assign (resid   82 and name N ) (resid   82 and name CA )  (resid   82 and name C )
(resid   83 and name N )  1.00  -35.00   20.00   2
!
assign (resid   83 and name N ) (resid   83 and name CA )  (resid   83 and name C )
(resid   84 and name N )  1.00  -35.00   20.00   2
!
assign (resid   84 and name N ) (resid   84 and name CA )  (resid   84 and name C )
(resid   85 and name N )  1.00  -35.00   20.00   2
```

```
!
assign (resid   85 and name N ) (resid   85 and name CA )  (resid   85 and name C )
(resid   86 and name N )  1.00  -35.00   20.00   2
!
assign (resid   86 and name N ) (resid   86 and name CA )  (resid   86 and name C )
(resid   87 and name N )  1.00  -35.00   20.00   2
!
assign (resid   87 and name N ) (resid   87 and name CA )  (resid   87 and name C )
(resid   88 and name N )  1.00  -35.00   20.00   2
!
assign (resid   88 and name N ) (resid   88 and name CA )  (resid   88 and name C )
(resid   89 and name N )  1.00  -35.00   20.00   2
!
assign (resid   89 and name N ) (resid   89 and name CA )  (resid   89 and name C )
(resid   90 and name N )  1.00  -35.00   20.00   2
!
assign (resid   90 and name N ) (resid   90 and name CA )  (resid   90 and name C )
(resid   91 and name N )  1.00  -35.00   20.00   2
!
assign (resid   91 and name N ) (resid   91 and name CA )  (resid   91 and name C )
(resid   92 and name N )  1.00  -35.00   20.00   2
!
assign (resid   92 and name N ) (resid   92 and name CA )  (resid   92 and name C )
(resid   93 and name N )  1.00  -35.00   20.00   2
!
assign (resid   94 and name N ) (resid   94 and name CA )  (resid   94 and name C )
(resid   95 and name N )  1.00  -35.00   40.00   2
!
assign (resid   96 and name N ) (resid   96 and name CA )  (resid   96 and name C )
(resid   97 and name N )  1.00  -35.00   40.00   2
!
assign (resid   99 and name N ) (resid   99 and name CA )  (resid   99 and name C )
(resid   100 and name N )  1.00  135.00   20.00   2
!
assign (resid   100 and name N ) (resid   100 and name CA )  (resid   100 and name C )
(resid   101 and name N )  1.00  135.00   20.00   2
!
assign (resid   101 and name N ) (resid   101 and name CA )  (resid   101 and name C )
(resid   102 and name N )  1.00  135.00   20.00   2
!
assign (resid   102 and name N ) (resid   102 and name CA )  (resid   102 and name C )
(resid   103 and name N )  1.00  -35.00   20.00   2
!
assign (resid   103 and name N ) (resid   103 and name CA )  (resid   103 and name C )
(resid   104 and name N )  1.00  -35.00   20.00   2
!
assign (resid   104 and name N ) (resid   104 and name CA )  (resid   104 and name C )
(resid   105 and name N )  1.00  -35.00   20.00   2
!
assign (resid   105 and name N ) (resid   105 and name CA )  (resid   105 and name C )
(resid   106 and name N )  1.00  -35.00   20.00   2
!
assign (resid   106 and name N ) (resid   106 and name CA )  (resid   106 and name C )
(resid   107 and name N )  1.00  -35.00   20.00   2
!
assign (resid   107 and name N ) (resid   107 and name CA )  (resid   107 and name C )
(resid   108 and name N )  1.00  -35.00   20.00   2
!
assign (resid   108 and name N ) (resid   108 and name CA )  (resid   108 and name C )
(resid   109 and name N )  1.00  -35.00   20.00   2
!
assign (resid   109 and name N ) (resid   109 and name CA )  (resid   109 and name C )
(resid   110 and name N )  1.00  -35.00   20.00   2
!
assign (resid   111 and name N ) (resid   111 and name CA )  (resid   111 and name C )
(resid   112 and name N )  1.00  -35.00   20.00   2
!
assign (resid   116 and name N ) (resid   116 and name CA )  (resid   116 and name C )
(resid   117 and name N )  1.00  135.00   20.00   2
!
```

```
assign (resid   118 and name N ) (resid   118 and name CA )  (resid   118 and name C )
(resid   119 and name N )  1.00  -35.00   20.00   2
!
assign (resid   119 and name N ) (resid   119 and name CA )  (resid   119 and name C )
(resid   120 and name N )  1.00  -35.00   20.00   2
!
assign (resid   120 and name N ) (resid   120 and name CA )  (resid   120 and name C )
(resid   121 and name N )  1.00  -35.00   20.00   2
!
assign (resid   121 and name N ) (resid   121 and name CA )  (resid   121 and name C )
(resid   122 and name N )  1.00  -35.00   20.00   2
!
assign (resid   122 and name N ) (resid   122 and name CA )  (resid   122 and name C )
(resid   123 and name N )  1.00  -35.00   20.00   2
!
assign (resid   123 and name N ) (resid   123 and name CA )  (resid   123 and name C )
(resid   124 and name N )  1.00  -35.00   20.00   2
!
assign (resid   124 and name N ) (resid   124 and name CA )  (resid   124 and name C )
(resid   125 and name N )  1.00  -35.00   20.00   2
!
assign (resid   125 and name N ) (resid   125 and name CA )  (resid   125 and name C )
(resid   126 and name N )  1.00  -35.00   20.00   2
!
assign (resid   126 and name N ) (resid   126 and name CA )  (resid   126 and name C )
(resid   127 and name N )  1.00  -35.00   20.00   2
!
assign (resid   127 and name N ) (resid   127 and name CA )  (resid   127 and name C )
(resid   128 and name N )  1.00  -35.00   20.00   2
!
assign (resid   130 and name N ) (resid   130 and name CA )  (resid   130 and name C )
(resid   131 and name N )  1.00  -35.00   40.00   2
!
assign (resid   135 and name N ) (resid   135 and name CA )  (resid   135 and name C )
(resid   136 and name N )  1.00  135.00   20.00   2
!
assign (resid   136 and name N ) (resid   136 and name CA )  (resid   136 and name C )
(resid   137 and name N )  1.00  135.00   20.00   2
!
assign (resid   137 and name N ) (resid   137 and name CA )  (resid   137 and name C )
(resid   138 and name N )  1.00  135.00   20.00   2
!
assign (resid   138 and name N ) (resid   138 and name CA )  (resid   138 and name C )
(resid   139 and name N )  1.00  -35.00   20.00   2
!
assign (resid   139 and name N ) (resid   139 and name CA )  (resid   139 and name C )
(resid   140 and name N )  1.00  -35.00   20.00   2
!
assign (resid   140 and name N ) (resid   140 and name CA )  (resid   140 and name C )
(resid   141 and name N )  1.00  -35.00   20.00   2
!
assign (resid   141 and name N ) (resid   141 and name CA )  (resid   141 and name C )
(resid   142 and name N )  1.00  -35.00   20.00   2
!
assign (resid   142 and name N ) (resid   142 and name CA )  (resid   142 and name C )
(resid   143 and name N )  1.00  -35.00   20.00   2
!
assign (resid   143 and name N ) (resid   143 and name CA )  (resid   143 and name C )
(resid   144 and name N )  1.00  -35.00   20.00   2
!
assign (resid   144 and name N ) (resid   144 and name CA )  (resid   144 and name C )
(resid   145 and name N )  1.00  -35.00   20.00   2
!
assign (resid   145 and name N ) (resid   145 and name CA )  (resid   145 and name C )
(resid   146 and name N )  1.00  -35.00   20.00   2
!
assign (resid   146 and name N ) (resid   146 and name CA )  (resid   146 and name C )
(resid   147 and name N )  1.00  -35.00   20.00   2

!   ACaM side chain "chi1" angles
```

```
!      L4
assign (resid  4 and name  N) (resid  4 and name CA )
       (resid  4 and name CB) (resid  4 and name HB2) 1.00    180.00     30.00 2
!      T5
assign (resid  5 and name  N) (resid  5 and name CA)
       (resid  5 and name CB) (resid  5 and name HB) 1.00    180.00     30.00 2
!      E7
assign (resid  7 and name  N) (resid  7 and name CA)
       (resid  7 and name CB) (resid  7 and name HB2) 1.00    180.00     30.00 2
!      Q8
assign (resid  8 and name  N) (resid  8 and name CA)
       (resid  8 and name CB) (resid  8 and name HB2) 1.00    180.00     30.00 2
!      I9
assign (resid  9 and name  N) (resid  9 and name CA)
       (resid  9 and name CB) (resid  9 and name HB) 1.00    180.00     30.00 2
!      F12
assign (resid 12 and name  N) (resid 12 and name CA)
       (resid 12 and name CB) (resid 12 and name HB2) 1.00    180.00     30.00 2
!      D14
assign (resid 14 and name  N) (resid 14 and name CA)
       (resid 14 and name CB) (resid 14 and name HB2) 1.00    180.00     30.00 2
!      V17
assign (resid 17 and name  N) (resid 17 and name CA)
       (resid 17 and name CB) (resid 17 and name HB) 1.00    180.00     30.00 2
!      Q18
assign (resid 18 and name  N) (resid 18 and name CA)
       (resid 18 and name CB) (resid 18 and name HB2) 1.00    180.00     30.00 2
!      F19
assign (resid 19 and name  N) (resid 19 and name CA)
       (resid 19 and name CB) (resid 19 and name HB3) 1.00    180.00     30.00 2
!      E22
assign (resid 22 and name  N) (resid 22 and name CA)
       (resid 22 and name CB) (resid 22 and name HB3) 1.00    180.00     30.00 2
!      T24
assign (resid 24 and name  N) (resid 24 and name CA)
       (resid 24 and name CB) (resid 24 and name HB) 1.00    180.00     30.00 2
!      K26
assign (resid 26 and name  N) (resid 26 and name CA)
       (resid 26 and name CB) (resid 26 and name HB3) 1.00    180.00     30.00 2
!      I27
assign (resid 27 and name  N) (resid 27 and name CA)
       (resid 27 and name CB) (resid 27 and name HB) 1.00    180.00     30.00 2
!      R30
assign (resid 30 and name  N) (resid 30 and name CA)
       (resid 30 and name CB) (resid 30 and name HB3) 1.00    180.00     30.00 2
!      E31
assign (resid 31 and name  N) (resid 31 and name CA)
       (resid 31 and name CB) (resid 31 and name HB3) 1.00    180.00     30.00 2
!      L35
assign (resid 35 and name  N) (resid 35 and name CA)
       (resid 35 and name CB) (resid 35 and name HB3) 1.00    180.00     30.00 2
!      M36
assign (resid 36 and name  N) (resid 36 and name CA)
       (resid 36 and name CB) (resid 36 and name HB2) 1.00    180.00     30.00 2
!      R37
assign (resid 37 and name  N) (resid 37 and name CA)
       (resid 37 and name CB) (resid 37 and name HB3) 1.00    180.00     30.00 2
!      T38
assign (resid 38 and name  N) (resid 38 and name CA)
       (resid 38 and name CB) (resid 38 and name HB) 1.00    180.00     30.00 2
!      L39
assign (resid 39 and name  N) (resid 39 and name CA)
       (resid 39 and name CB) (resid 39 and name HB3) 1.00    180.00     30.00 2
!      Q41
assign (resid 41 and name  N) (resid 41 and name CA)
       (resid 41 and name CB) (resid 41 and name HB3) 1.00    180.00     30.00 2
!      T44
assign (resid 44 and name  N) (resid 44 and name CA)
       (resid 44 and name CB) (resid 44 and name HB) 1.00    180.00     30.00 2
!      E47
assign (resid 47 and name  N) (resid 47 and name CA)
```

```
      (resid 47 and name CB) (resid 47 and name HB2) 1.00    180.00     30.00 2
!      Q49
assign (resid 49 and name  N) (resid 49 and name CA)
      (resid 49 and name CB) (resid 49 and name HB2) 1.00    180.00     30.00 2
!      D50
assign (resid 50 and name  N) (resid 50 and name CA)
      (resid 50 and name CB) (resid 50 and name HB2) 1.00    180.00     30.00 2
!      L51
assign (resid 51 and name  N) (resid 51 and name CA)
      (resid 51 and name CB) (resid 51 and name HB2) 1.00    180.00     30.00 2
!      I52
assign (resid 52 and name  N) (resid 52 and name CA)
      (resid 52 and name CB) (resid 52 and name HB) 1.00    180.00     30.00 2
!      E56
assign (resid 56 and name  N) (resid 56 and name CA)
      (resid 56 and name CB) (resid 56 and name HB2) 1.00    180.00     30.00 2
!      Q62
assign (resid 62 and name  N) (resid 62 and name CA)
      (resid 62 and name CB) (resid 62 and name HB3) 1.00    180.00     30.00 2
!      N64
assign (resid 64 and name  N) (resid 64 and name CA)
      (resid 64 and name CB) (resid 64 and name HB3) 1.00    180.00     30.00 2
!      T66
assign (resid 66 and name  N) (resid 66 and name CA)
      (resid 66 and name CB) (resid 66 and name HB) 1.00    180.00     30.00 2
!      E67
assign (resid 67 and name  N) (resid 67 and name CA)
      (resid 67 and name CB) (resid 67 and name HB3) 1.00    180.00     30.00 2
!      C69
assign (resid 69 and name  N) (resid 69 and name CA)
      (resid 69 and name CB) (resid 69 and name HB2) 1.00    180.00     30.00 2
!      I71
assign (resid 71 and name  N) (resid 71 and name CA)
      (resid 71 and name CB) (resid 71 and name HB) 1.00    180.00     30.00 2
!      M72
assign (resid 72 and name  N) (resid 72 and name CA)
      (resid 72 and name CB) (resid 72 and name HB2) 1.00    180.00     30.00 2
!      K74
assign (resid 74 and name  N) (resid 74 and name CA)
      (resid 74 and name CB) (resid 74 and name HB3) 1.00    180.00     30.00 2
!      Q75
assign (resid 75 and name  N) (resid 75 and name CA)
      (resid 75 and name CB) (resid 75 and name HB2) 1.00    180.00     30.00 2
!      M76
assign (resid 76 and name  N) (resid 76 and name CA)
      (resid 76 and name CB) (resid 76 and name HB3) 1.00    180.00     30.00 2
!      D80
assign (resid 80 and name  N) (resid 80 and name CA)
      (resid 80 and name CB) (resid 80 and name HB2) 1.00    180.00     30.00 2
!      E83
assign (resid 83 and name  N) (resid 83 and name CA)
      (resid 83 and name CB) (resid 83 and name HB2) 1.00    180.00     30.00 2
!      E84
assign (resid 84 and name  N) (resid 84 and name CA)
      (resid 84 and name CB) (resid 84 and name HB2) 1.00    180.00     30.00 2
!      M85
assign (resid 85 and name  N) (resid 85 and name CA)
      (resid 85 and name CB) (resid 85 and name HB2) 1.00    180.00     30.00 2
!      R86
assign (resid 86 and name  N) (resid 86 and name CA)
      (resid 86 and name CB) (resid 86 and name HB2) 1.00    180.00     30.00 2
!      E87
assign (resid 87 and name  N) (resid 87 and name CA)
      (resid 87 and name CB) (resid 87 and name HB2) 1.00    180.00     30.00 2
!      I91
assign (resid 91 and name  N) (resid 91 and name CA)
      (resid 91 and name CB) (resid 91 and name HB) 1.00    180.00     30.00 2
!      R94
assign (resid 94 and name  N) (resid 94 and name CA)
      (resid 94 and name CB) (resid 94 and name HB3) 1.00    180.00     30.00 2
!      D95
```

```
assign (resid 95 and name  N) (resid 95 and name CA)
      (resid 95 and name CB) (resid 95 and name HB3) 1.00    180.00     30.00 2
!      D97
assign (resid 97 and name  N) (resid 97 and name CA)
      (resid 97 and name CB) (resid 97 and name HB3) 1.00    180.00     30.00 2
!      F99
assign (resid 99 and name  N) (resid 99 and name CA)
      (resid 99 and name CB) (resid 99 and name HB2) 1.00    180.00     30.00 2
!      S101
assign (resid 101 and name  N) (resid 101 and name CA)
      (resid 101 and name CB) (resid 101 and name HB3) 1.00    180.00     30.00 2
!      E104
assign (resid 104 and name  N) (resid 104 and name CA)
      (resid 104 and name CB) (resid 104 and name HB3) 1.00    180.00     30.00 2
!      F107
assign (resid 107 and name  N) (resid 107 and name CA)
      (resid 107 and name CB) (resid 107 and name HB3) 1.00    180.00     30.00 2
!      I110
assign (resid 110 and name  N) (resid 110 and name CA)
      (resid 110 and name CB) (resid 110 and name HB) 1.00    180.00     30.00 2
!      L112
assign (resid 112 and name  N) (resid 112 and name CA)
      (resid 112 and name CB) (resid 112 and name HB2) 1.00    180.00     30.00 2
!      E114
assign (resid 114 and name  N) (resid 114 and name CA)
      (resid 114 and name CB) (resid 114 and name HB3) 1.00    180.00     30.00 2
!      K115
assign (resid 115 and name  N) (resid 115 and name CA)
      (resid 115 and name CB) (resid 115 and name HB3) 1.00    180.00     30.00 2
!      V116
assign (resid 116 and name  N) (resid 116 and name CA)
      (resid 116 and name CB) (resid 116 and name HB) 1.00    180.00     30.00 2
!      T117
assign (resid 117 and name  N) (resid 117 and name CA)
      (resid 117 and name CB) (resid 117 and name HB) 1.00    180.00     30.00 2
!      D118
assign (resid 118 and name  N) (resid 118 and name CA)
      (resid 118 and name CB) (resid 118 and name HB3) 1.00    180.00     30.00 2
!      E119
assign (resid 119 and name  N) (resid 119 and name CA)
      (resid 119 and name CB) (resid 119 and name HB2) 1.00    180.00     30.00 2
!      E120
assign (resid 120 and name  N) (resid 120 and name CA)
      (resid 120 and name CB) (resid 120 and name HB2) 1.00    180.00     30.00 2
!      I121
assign (resid 121 and name  N) (resid 121 and name CA)
      (resid 121 and name CB) (resid 121 and name HB) 1.00    180.00     30.00 2
!      D122
assign (resid 122 and name  N) (resid 122 and name CA)
      (resid 122 and name CB) (resid 122 and name HB2) 1.00    180.00     30.00 2
!      E123
assign (resid 123 and name  N) (resid 123 and name CA)
      (resid 123 and name CB) (resid 123 and name HB2) 1.00    180.00     30.00 2
!      M124
assign (resid 124 and name  N) (resid 124 and name CA)
      (resid 124 and name CB) (resid 124 and name HB2) 1.00    180.00     30.00 2
!      I125
assign (resid 125 and name  N) (resid 125 and name CA)
      (resid 125 and name CB) (resid 125 and name HB) 1.00    180.00     30.00 2
!      R126
assign (resid 126 and name  N) (resid 126 and name CA)
      (resid 126 and name CB) (resid 126 and name HB2) 1.00    180.00     30.00 2
!      F130
assign (resid 130 and name  N) (resid 130 and name CA)
      (resid 130 and name CB) (resid 130 and name HB3) 1.00    180.00     30.00 2
!      D131
assign (resid 131 and name  N) (resid 131 and name CA)
      (resid 131 and name CB) (resid 131 and name HB3) 1.00    180.00     30.00 2
!      D133
assign (resid 133 and name  N) (resid 133 and name CA)
      (resid 133 and name CB) (resid 133 and name HB3) 1.00    180.00     30.00 2
```

```
!      M135
assign (resid 135 and name  N) (resid 135 and name CA)
       (resid 135 and name CB) (resid 135 and name HB2) 1.00    180.00     30.00 2
!      E139
assign (resid 139 and name  N) (resid 139 and name CA)
       (resid 139 and name CB) (resid 139 and name HB2) 1.00    180.00     30.00 2
!      E140
assign (resid 140 and name  N) (resid 140 and name CA)
       (resid 140 and name CB) (resid 140 and name HB2) 1.00    180.00     30.00 2
!      V142
assign (resid 142 and name  N) (resid 142 and name CA)
       (resid 142 and name CB) (resid 142 and name HB) 1.00    180.00     30.00 2
!      Q147
assign (resid 147 and name  N) (resid 147 and name CA)
       (resid 147 and name CB) (resid 147 and name HB3) 1.00    180.00     30.00 2
!      K148
assign (resid 148 and name  N) (resid 148 and name CA)
       (resid 148 and name CB) (resid 148 and name HB3) 1.00    180.00     30.00 2
```

B.4 Stereoassignments for holo androcam

File name : "stereoassign.cns"

```
!$Revision: 2.1 $
!$Date: 2001/06/18 12:28:21 $
!$RCSfile: stereoassign.cns,v $
!!! SWAP
!!! methylene protons
for $id in id
(
{===>}   (name cb and resid 102)
{===>}or (name cb and resid 109)
{===>}or (name cb and resid 144)
)
loop Lmethy
    display aria revert  (bondedto (id $id) and name h*)  end
    display do (store1=0) (bondedto (id $id) and name h*)
end loop Lmethy
!
!!! isopropyle groups
!for $id in id (name ca and (
!{===>}resid 84
!))
!loop Vrev
!   display do (store1=0) (byresid (id $id) and (name cg1 or name cd1))
!    show (resn) (id $id)
!    if ($result eq VAL) then
!      evaluate ($name3 = cg1)
!      evaluate ($name4 = cg2)
!    elseif ($result eq LEU) then
!      evaluate ($name3 = cd1)
!      evaluate ($name4 = cd2)
!    end if
!    display aria revert (bondedto (byresid(id $id)
!    display and (name $name3 or name $name4)) and name h*) end
!end loop Vrev
!
!!! AS IS
!!! methylene protons
for $id in id
(
{===>}   (name cb and resid 4)
{===>}or (name cb and resid 7)
{===>}or (name cb and resid 8)
{===>}or (name cb and resid 12)
{===>}or (name cb and resid 14)
{===>}or (name cb and resid 18)
{===>}or (name cb and resid 19)
{===>}or (name cb and resid 26)
{===>}or (name cb and resid 30)
{===>}or (name cb and resid 31)
{===>}or (name cb and resid 36)
{===>}or (name cb and resid 37)
{===>}or (name cb and resid 39)
{===>}or (name cb and resid 41)
{===>}or (name cb and resid 43)
{===>}or (name cb and resid 47)
{===>}or (name cb and resid 49)
{===>}or (name cb and resid 50)
{===>}or (name cb and resid 51)
{===>}or (name cb and resid 56)
{===>}or (name cb and resid 64)
{===>}or (name cb and resid 69)
{===>}or (name cb and resid 72)
{===>}or (name cb and resid 74)
{===>}or (name cb and resid 76)
{===>}or (name cb and resid 80)
{===>}or (name cb and resid 85)
```

```
{===>}or (name cb and resid 92)
{===>}or (name cb and resid 94)
{===>}or (name cb and resid 95)
{===>}or (name cb and resid 97)
{===>}or (name cb and resid 99)
{===>}or (name cb and resid 101)
{===>}or (name cb and resid 112)
{===>}or (name cb and resid 114)
{===>}or (name cb and resid 118)
{===>}or (name cb and resid 119)
{===>}or (name cb and resid 120)
{===>}or (name cb and resid 122)
{===>}or (name cb and resid 123)
{===>}or (name cb and resid 124)
{===>}or (name cb and resid 126)
{===>}or (name cb and resid 133)
{===>}or (name cb and resid 135)
{===>}or (name cb and resid 139)
{===>}or (name cb and resid 140)
{===>}or (name cb and resid 147)
{===>}or (name cb and resid 148)
)
loop Lmethy
    display do (store1=0) (bondedto (id $id) and name h*)
end loop Lmethy ! typo in original ARIA file, changed by MKJ 2008-09-09
!
!!! isopropyle groups
!for $id in id (name ca and
!(
!{===>}   resid 27
!{===>}or resid 29
!{===>}or resid 46
!{===>}or resid 63
!{===>}or resid 37
!{===>}or resid 66
!))
!loop Vrev
! display do (store1=0) (byresid (id $id) and (name cg1 or name cd1))
!end loop Vrev
```

B.5 CSI input / output files for holo androcam

CSI input
File name : "csi.holoacam"

```
>Androcam (Drosophila Melanogaster)
>M. Ikura, L.E. Kay and A. Bax, Biochemistry 29:4659 (1990)

#       AA      HA      CA      CB      CO
1       M       0       0       0       0
2       S       4.35    57.9    63.0    177.0
3       E       4.29    55.6    29.5    174.7
4       L       4.58    53.3    42.5    177.7
5       T       4.42    59.4    70.3    175.3
6       E       3.90    59.0    28.4    179.4
7       E       4.10    59.3    28.1    179.2
8       Q       3.75    57.8    28.3    177.2
9       I       3.37    64.9    36.8    177.8
10      A       4.11    54.5    17.2    180.4
11      E       4.16    58.4    28.6    180.4
12      F       4.90    56.7    35.7    178.5
13      K       3.70    59.0    30.6    177.6
14      D       4.40    56.4    39.9    178.4
15      A       4.36    53.9    18.7    179.0
16      F       3.78    61.5    40.1    177.8
17      V       3.89    64.6    30.8    178.5
18      Q       3.93    56.9    27.1    177.0
19      F       4.25    57.9    38.9    174.9
20      D       4.91    51.4    39.0    176.8
21      K       4.03    57.9    31.4    178.6
22      E       4.38    55.3    29.2    177.2
23      G       3.89    46.5    0       176.1
24      T       4.31    61.4    69.8    176.8
25      G       4.06    44.6    0       173.6
26      K       5.42    53.8    36.2    174.6
27      I       4.54    57.4    40.7    175.0
28      A       4.78    50.5    18.5    179.6
29      T       3.59    64.9    66.4    177.3
30      R       4.23    57.3    28.6    177.0
31      E       4.62    55.0    28.9    175.9
32      L       3.67    57.7    41.3    177.9
33      G       3.62    47.3    0       175.2
34      T       3.83    65.2    67.5    176.8
35      L       3.67    57.5    39.4    178.7
36      M       3.76    60.4    32.0    178.7
37      R       4.60    58.5    29.5    181.6
38      T       4.21    65.3    68.3    175.5
39      L       4.58    53.5    41.9    177.0
40      G       4.03    45.2    0       174.4
41      Q       4.42    52.7    29.4    173.8
42      N       5.17    50.2    38.6    172.0
43      P       4.72    61.5    30.7    177.5
44      T       4.37    60.1    69.9    175.0
45      E       3.99    59.2    28.2    179.1
46      A       4.07    54.2    17.5    180.6
47      E       4.01    57.9    29.0    180.0
48      L       3.93    57.2    41.0    178.2
49      Q       3.89    58.0    27.1    178.8
50      D       4.48    56.4    39.7    178.7
51      L       4.06    56.9    41.6    179.8
52      I       3.70    63.7    37.7    177.1
53      A       4.11    54.5    17.5    180.3
54      E       4.10    57.9    28.5    178.1
55      A       4.30    53.0    17.9    180.1
56      E       3.99    57.8    28.4    177.3
57      N       4.67    53.6    38.4    0
58      N       4.80    53.1    38.8    176.9
59      N       4.89    52.6    38.7    179.9
```

60	N	4.62	53.1	37.4	0
61	G	3.94	45.6	0	174.4
62	Q	5.34	53.4	32.6	174.5
63	L	5.00	52.8	45.3	175.9
64	N	5.66	50.1	38.4	175.8
65	F	3.62	61.2	38.2	177.4
66	T	3.60	66.1	67.5	177.9
67	E	3.98	58.1	29.0	180.1
68	F	4.09	60.6	38.3	176.3
69	C	3.35	64.2	25.7	176.5
70	G	3.77	46.2	0	176.5
71	I	3.59	64.4	37.5	178.1
72	M	3.97	55.2	29.5	178.6
73	A	3.99	54.4	17.1	180.1
74	K	3.97	58.3	31.6	178.6
75	Q	4.01	56.4	27.3	177.8
76	M	4.20	56.3	32.5	176.8
77	R	4.27	56.4	29.8	177.9
78	E	4.34	56.2	29.2	177.1
79	T	4.31	61.1	68.9	174.5
80	D	4.75	53.9	40.3	177.0
81	T	4.32	62.2	69.1	175.6
82	E	4.18	58.3	28.6	177.9
83	E	4.02	58.8	28.5	179.1
84	E	4.13	58.6	28.7	179.9
85	M	4.51	58.1	32.1	178.6
86	R	4.09	58.7	28.9	179.7
87	E	4.07	58.2	28.4	179.0
88	A	4.21	54.3	17.1	179.2
89	F	3.09	61.2	38.6	176.6
90	K	3.87	58.2	31.8	178.1
91	I	3.59	62.8	36.5	177.4
92	F	4.24	59.1	39.5	176.8
93	D	4.50	51.3	37.5	177.0
94	R	3.86	58.0	37.5	178.2
95	D	4.55	52.1	39.0	177.9
96	G	3.81	46.6	0	175.2
97	D	4.47	53.2	39.6	176.8
98	G	3.72	44.2	0	172.5
99	F	5.17	55.3	43.6	174.7
100	I	4.77	59.7	38.0	175.6
101	S	5.10	55.0	62.7	173.6
102	P	4.11	65.6	30.6	178.4
103	A	4.10	54.3	17.8	181.6
104	E	4.07	58.6	28.4	178.8
105	L	4.13	57.5	41.3	178.3
106	R	3.76	59.4	29.8	177.1
107	F	4.09	60.7	38.8	178.1
108	V	3.42	65.6	30.8	177.9
109	M	4.10	58.0	31.0	178.8
110	I	3.97	63.3	36.5	180.4
111	N	4.31	55.4	0	0
112	L	4.31	54.5	41.5	177.7
113	G	4.15	44.6	0	174.6
114	E	4.32	54.7	29.6	175.9
115	K	4.37	54.8	30.6	175.8
116	V	4.66	59.2	33.8	175.6
117	T	4.59	59.3	70.8	175.4
118	D	4.28	57.2	39.1	178.5
119	E	4.12	59.2	28.2	179.2
120	E	3.97	58.3	29.1	179.8
121	I	3.81	62.5	35.4	177.3
122	D	4.33	56.9	39.7	179.1
123	E	4.01	58.5	28.8	178.1
124	M	4.17	58.5	32.8	179.1
125	I	3.56	63.5	36.2	176.9
126	R	3.93	58.9	29.3	179.5
127	E	3.91	57.8	29.2	176.5
128	A	4.14	50.9	19.9	175.7
129	D	4.55	52.1	38.7	177.5
130	F	4.32	58.7	38.1	177.8

```
131     D       4.63    52.5    39.0    178.3
132     G       3.84    46.7    0       175.2
133     D       4.44    52.8    39.2    177.4
134     G       3.66    44.8    0       173.0
135     M       4.89    51.6    36.5    175.0
136     I       5.40    57.5    37.2    176.2
137     N       5.22    50.3    37.1    175.0
138     Y       3.55    62.1    37.0    175.9
139     E       3.67    59.7    28.3    180.5
140     E       4.12    57.9    28.9    178.3
141     F       4.07    60.5    38.7    176.2
142     V       3.23    65.6    30.8    179.4
143     W       4.03    61.2    27.8    178.5
144     M       3.72    58.8    33.3    179.1
145     I       3.84    60.9    36.5    177.5
146     S       4.30    58.8    62.8    174.3
147     Q       3.95    54.5    27.8    175.1
148     K       4.00    56.8    32.4    181.4
```

CSI output
File name : "holo_ACaM.out"

```
#
#
################################################################################
#  Program...:                          CSI (c)
#  Version...:                           2.0
#  Location..:                    University of Alberta
#                              Protein Engineering Network of
#                                   Centres of Excellence
#  Input.....:                      /homes/mkjoshi/androcam/csiACaM/lib/csi/SAMPLE/csi.acamraw
#  Date......:                      Wed Nov 28 13:29:44 2007
################################################################################
#
#
#     A       HA       CA       CO       CB       Consensus
#
1     M       0 C      0 C      0 C      0 C          0 C
2     S       0 C      0 C      0 C      0 C          0 C
3     E       0 C      0 C      0 C      0 C          0 C
4     L       1 C     -1 C      1 C      0 C          0 C
5     T       0 C     -1 C      0 H      1 C          0 C
6     E      -1 H      1 H      1 H     -1 C         -1 H
7     E      -1 H      1 H      1 H     -1 C         -1 H
8     Q      -1 H      1 H      1 H     -1 C         -1 H
9     I      -1 H      1 H      1 H     -1 C         -1 H
10    A      -1 H      1 H      1 H     -1 C         -1 H
11    E      -1 H      1 H      1 H     -1 C         -1 H
12    F       1 C     -1 C      1 H     -1 C          0 C
13    K      -1 C      1 H      1 H     -1 C         -1 H
14    D      -1 C      1 H      1 H     -1 C         -1 H
15    A       0 C      1 H      1 H      0 C         -1 H
16    F      -1 C      1 H      1 H      1 C         -1 H
17    V       0 C      1 H      1 H     -1 C         -1 H
18    Q      -1 C      0 C      1 H     -1 C          0 C
19    F      -1 C      0 C     -1 C      0 C          0 C
20    D       1 C     -1 C      0 C     -1 C          0 C
21    K      -1 C      1 C      1 H     -1 C          0 C
22    E       0 C     -1 C      1 H      0 C          0 C
23    G       0 C      1 C      1 H      0 C          0 C
24    T       0 C     -1 B      1 H      1 C          0 C
25    G       0 C      0 B      0 C      0 C          0 C
26    K       1 B     -1 B     -1 C      1 C          1 B
27    I       1 B     -1 B     -1 C      1 C          1 B
28    A       1 B     -1 B      1 H      0 C          1 B
29    T      -1 C      1 C      1 H     -1 C          0 C
30    R      -1 C      1 C      1 H     -1 C          0 C
31    E       1 C     -1 C      0 H     -1 C          0 C
32    L      -1 H      1 H      1 H      0 C         -1 H
33    G      -1 H      1 H      1 H     -1 C         -1 H
```

34	T	-1 H	1 H	1 H	0 C	-1 H
35	L	-1 H	1 H	1 H	-1 C	-1 H
36	M	-1 H	1 H	1 H	-1 C	-1 H
37	R	1 C	1 H	1 H	-1 C	-1 H
38	T	-1 C	1 H	0 C	0 C	0 C
39	L	1 C	-1 C	0 C	0 C	0 C
40	G	0 C	0 C	1 C	0 C	0 C
41	Q	0 C	-1 C	-1 C	-1 C	0 C
42	N	1 C	-1 C	-1 C	0 C	0 C
43	P	1 C	0 C	0 C	0 C	0 C
44	T	0 C	-1 C	0 C	1 C	0 C
45	E	-1 H	1 H	1 H	-1 C	-1 H
46	A	-1 H	1 H	1 H	-1 C	-1 H
47	E	-1 H	1 H	1 H	0 C	-1 H
48	L	-1 H	1 H	1 H	-1 C	-1 H
49	Q	-1 H	1 H	1 H	-1 C	-1 H
50	D	-1 H	1 H	1 H	-1 C	-1 H
51	L	-1 H	1 H	1 H	0 C	-1 H
52	I	-1 H	1 H	0 H	0 C	-1 H
53	A	-1 H	1 H	1 H	-1 C	-1 H
54	E	-1 H	1 H	1 H	-1 C	-1 H
55	A	0 H	0 H	1 H	-1 C	-1 H
56	E	-1 C	1 C	1 H	-1 C	0 C
57	N	0 C	0 C	0 C	0 C	0 C
58	N	0 C	0 C	0 C	0 C	0 C
59	N	0 C	0 C	0 C	0 C	0 C
60	N	0 C	0 C	0 C	0 C	0 C
61	G	0 C	0 C	1 C	0 C	0 C
62	Q	1 B	-1 B	-1 C	1 C	1 B
63	L	1 B	-1 B	-1 C	1 C	1 B
64	N	1 B	-1 B	1 H	0 C	1 B
65	F	-1 H	1 H	1 H	-1 C	-1 H
66	T	-1 H	1 H	1 H	0 C	-1 H
67	E	-1 H	1 H	1 H	0 C	-1 H
68	F	-1 H	1 H	1 H	-1 C	-1 H
69	C	-1 H	1 H	1 H	-1 C	-1 H
70	G	-1 H	1 H	1 H	-1 C	-1 H
71	I	-1 H	1 H	1 H	0 C	-1 H
72	M	-1 H	-1 C	1 H	-1 C	-1 H
73	A	-1 H	1 C	1 H	-1 C	-1 H
74	K	-1 H	1 C	1 H	0 C	-1 H
75	Q	-1 H	0 C	1 H	-1 C	-1 H
76	M	-1 H	0 C	1 H	0 C	-1 H
77	R	-1 H	0 C	1 H	0 C	-1 H
78	E	0 C	0 C	0 C	0 C	0 C
79	T	0 C	-1 C	-1 C	1 C	0 C
80	D	0 C	0 C	0 C	0 C	0 C
81	T	0 C	-1 C	0 C	1 C	0 C
82	E	0 C	0 C	0 C	0 C	0 C
83	E	0 C	0 C	0 C	0 C	0 C
84	E	0 C	0 C	0 C	0 C	0 C
85	M	0 C	0 C	0 C	0 C	0 C
86	R	-1 H	1 H	1 H	-1 C	-1 H
87	E	-1 H	1 H	1 H	-1 C	-1 H
88	A	-1 H	1 H	1 H	-1 C	-1 H
89	F	-1 H	1 H	1 H	0 C	-1 H
90	K	-1 H	1 H	1 H	0 C	-1 H
91	I	-1 H	0 H	0 H	-1 C	-1 H
92	F	-1 H	1 H	1 C	0 C	-1 H
93	D	-1 H	-1 C	0 H	-1 C	-1 H
94	R	-1 H	1 C	1 H	1 C	-1 H
95	D	-1 H	-1 C	1 H	-1 C	-1 H
96	G	-1 H	1 C	1 H	-1 C	-1 H
97	D	-1 H	-1 B	0 C	-1 C	0 C
98	G	-1 H	-1 B	-1 B	-1 C	0 C
99	F	1 B	-1 B	-1 B	1 C	1 B
100	I	1 B	-1 B	-1 B	0 C	1 B
101	S	1 B	-1 B	0 C	0 C	1 B
102	P	-1 H	0 C	0 C	0 C	0 C
103	A	-1 H	1 H	1 H	-1 C	-1 H
104	E	-1 H	1 H	1 H	-1 C	-1 H

```
105   L       0 H      1 H      1 H      0 C        -1 H
106   R      -1 H      1 H      1 H      0 C        -1 H
107   F      -1 H      1 H      1 H      0 C        -1 H
108   V      -1 H      1 H      1 H     -1 C        -1 H
109   M       0 C      0 C      0 C      0 C         0 C
110   I       0 C      0 C      0 C      0 C         0 C
111   N       0 C      0 C      0 C      0 C         0 C
112   L       1 C     -1 C     -1 C      0 C         0 C
113   G       1 C      0 B      1 C      1 C         0 C
114   E       0 C     -1 B      0 C      0 C         0 C
115   K       0 C     -1 B     -1 C     -1 C         0 C
116   V       1 C     -1 B     -1 C      1 C         0 C
117   T       1 C     -1 B      0 C      1 C         0 C
118   D      -1 H      1 H      1 H     -1 C        -1 H
119   E      -1 H      1 H      1 H     -1 C        -1 H
120   E      -1 H      1 H      1 H      0 C        -1 H
121   I      -1 H      0 H      0 H     -1 C        -1 H
122   D      -1 H      1 H      1 H     -1 C        -1 H
123   E      -1 H      1 H      1 H     -1 C        -1 H
124   M      -1 H      1 H      1 H      0 C        -1 H
125   I      -1 H      1 H      0 H     -1 C        -1 H
126   R      -1 H      1 H      1 C     -1 C        -1 H
127   E      -1 H      1 H      0 C      0 C        -1 H
128   A      -1 H     -1 C     -1 C      1 C        _0 C
129   D      -1 H     -1 C      0 C     -1 C         0 C
130   F      -1 H      1 C      1 C     -1 C         0 C
131   D      -1 H     -1 C      1 C     -1 C         0 C
132   G      -1 H      1 C      1 C     -1 C         0 C
133   D      -1 H     -1 B      0 C     -1 C         0 C
134   G      -1 H      0 B     -1 B     -1 C         0 C
135   M       1 B     -1 B     -1 B      1 C         1 B
136   I       1 B     -1 B     -1 B      0 C         1 B
137   N       1 B     -1 B      0 C     -1 C         1 B
138   Y      -1 H      1 H      0 C     -1 C        -1 H
139   E      -1 H      1 H      1 H     -1 C        -1 H
140   E      -1 H      1 H      1 H     -1 C        -1 H
141   F      -1 H      1 H      0 H      0 C        -1 H
142   V      -1 H      1 H      1 H     -1 C        -1 H
143   W      -1 H      1 H      1 H      0 C        -1 H
144   M      -1 H      1 H      1 H      0 C        -1 H
145   I      -1 H     -1 C      1 H     -1 C        -1 H
146   S      -1 H      0 C      1 H      0 C        -1 H
147   Q      -1 H     -1 C     -1 C     -1 C         0 C
148   K       0 C      0 C      0 C      0 C         0 C
#
#
#
#############################################################################
#
#                    Secondary Structure Summary
#
#
#
#
#       HA       |       CA       |       CO       |       CB       | Consensus   |
#-----------------------------------------------------------------------------------
# C   1 -   5    | C   1 -   5    | C   1 -   4    | C   1 - 148    | C   0 -   0 |
# H   6 -  11    | H   6 -  11    | H   5 -  18    |                | H   0 -   0 |
# C  12 -  25    | C  12 -  12    | C  19 -  20    |                | C   0 -   0 |
# B  26 -  28    | H  13 -  17    | H  21 -  24    |                | H   0 -   0 |
# C  29 -  31    | C  18 -  23    | C  25 -  27    |                | C   0 -   0 |
# H  32 -  36    | B  24 -  28    | H  28 -  37    |                | B   0 -   0 |
# C  37 -  44    | C  29 -  31    | C  38 -  44    |                | C   0 -   0 |
# H  45 -  55    | H  32 -  38    | H  45 -  56    |                | H   0 -   0 |
# C  56 -  61    | C  39 -  44    | C  57 -  63    |                | C   0 -   0 |
# B  62 -  64    | H  45 -  55    | H  64 -  77    |                | H   0 -   0 |
# H  65 -  77    | C  56 -  61    | C  78 -  85    |                | C   0 -   0 |
# C  78 -  85    | B  62 -  64    | H  86 -  91    |                | B   0 -   0 |
# H  86 -  98    | H  65 -  71    | C  92 -  92    |                | H   0 -   0 |
# B  99 - 101    | C  72 -  85    | H  93 -  96    |                | C   0 -   0 |
# H 102 - 108    | H  86 -  92    | C  97 -  97    |                | H   0 -   0 |
```

```
# C 109 - 117  | C  93 -  96  | B  98 - 100  |              | C   0 -   0  |
# H 118 - 134  | B  97 - 101  | C 101 - 102  |              | B   0 -   0  |
# B 135 - 137  | C 102 - 102  | H 103 - 108  |              | C   0 -   0  |
# H 138 - 147  | H 103 - 108  | C 109 - 117  |              | H   0 -   0  |
# C 148 - 148  | C 109 - 112  | H 118 - 125  |              | C   0 -   0  |
#              | B 113 - 117  | C 126 - 133  |              | H   0 -   0  |
#              | H 118 - 127  | B 134 - 136  |              | C   0 -   0  |
#              | C 128 - 132  | C 137 - 138  |              | B   0 -   0  |
#              | B 133 - 137  | H 139 - 146  |              | H   0 -   0  |
#              | H 138 - 144  | C 147 - 148  |              | C   0 -   0  |
#              | C 145 - 148  |              |              |              |
#
```

APPENDIX C (apoN androcam)

C.1 Chemical shifts for apoN androcam

Group	Atom	Shift
M1	C	177.06
M1	CA	55.68
M1	CB	29.44
M1	CE	17.34
M1	CG	33.67
M1	HA	4.21
M1	HB2	1.86
M1	HB3	1.94
M1	HE#	2.05
M1	HG#	2.20
S2	C	174.66
S2	CA	58.79
S2	CB	63.99
S2	H	8.19
S2	HA	4.35
S2	HB#	3.79
S2	N	121.66
E3	C	176.33
E3	CA	56.10
E3	CB	30.71
E3	CG	36.18
E3	H	8.53
E3	HA	4.36
E3	HB2	1.85
E3	HB3	2.04
E3	HG2	2.23
E3	HG3	2.25
E3	N	121.82
L4	C	177.86
L4	CA	54.37
L4	CB	43.16
L4	CD1	23.45
L4	CD2	26.50
L4	CG	27.00
L4	H	8.30
L4	HA	4.56
L4	HB2	1.26
L4	HB3	1.65
L4	HD1#	0.81
L4	HD2#	0.88
L4	HG	1.70
L4	N	121.99
T5	C	175.40
T5	CA	60.25
T5	CB	71.25
T5	CG2	21.68
T5	H	8.76
T5	HA	4.42
T5	HB	4.74
T5	HG2#	1.29
T5	N	113.55
E6	C	179.47
E6	CA	59.90
E6	CB	29.30
E6	CG	36.67
E6	H	8.98
E6	HA	3.89
E6	HB#	2.00
E6	HG2	2.28
E6	HG3	2.37
E6	N	120.26
E7	C	179.35
E7	CA	60.18
E7	CB	28.97
E7	CG	37.11
E7	H	8.68
E7	HA	4.00
E7	HB2	1.90
E7	HB3	2.01
E7	HG2	2.24
E7	HG3	2.36
E7	N	119.41
Q8	C	177.28
Q8	CA	58.71
Q8	CB	29.16
Q8	CG	34.94
Q8	H	7.68
Q8	HA	3.75
Q8	HB2	1.16
Q8	HB3	2.13
Q8	HE21	6.64
Q8	HE22	7.63
Q8	HG2	2.16
Q8	HG3	2.25
Q8	N	120.76
Q8	NE2	110.98
I9	C	177.84
I9	CA	65.75
I9	CB	37.60
I9	CD1	12.72
I9	CG1	30.15
I9	CG2	17.62
I9	H	8.08
I9	HA	3.36
I9	HB	1.83
I9	HD1#	0.75
I9	HG12	0.99
I9	HG13	1.61
I9	HG2#	1.10
I9	N	118.13
A10	C	180.41
A10	CA	55.35
A10	CB	17.95
A10	H	7.83
A10	HA	4.10
A10	HB#	1.47
A10	N	120.76
E11	C	180.40
E11	CA	59.32
E11	CB	29.49
E11	CG	36.28
E11	H	7.70
E11	HA	4.15
E11	HB#	2.16
E11	HG#	2.47
E11	N	119.85
F12	C	178.51
F12	CA	57.58
F12	CB	36.58
F12	CD#	130.09
F12	CE#	131.40
F12	CZ	128.89
F12	H	9.05
F12	HA	4.89
F12	HB2	3.57
F12	HB3	3.91
F12	HD#	7.20
F12	HE#	7.29
F12	HZ	7.11
F12	N	120.25
K13	C	177.63
K13	CA	59.85
K13	CB	31.38
K13	CD	28.28
K13	CE	41.66
K13	CG	24.41
K13	H	9.13
K13	HA	3.69
K13	HB2	1.64
K13	HB3	1.92
K13	HD2	1.05
K13	HD3	1.19
K13	HE#	2.51
K13	HG2	0.76
K13	HG3	1.16
K13	N	122.74
D14	C	178.39
D14	CA	57.29
D14	CB	40.75
D14	H	7.78
D14	HA	4.40
D14	HB2	2.69
D14	HB3	2.79
D14	N	117.81
A15	C	179.02
A15	CA	54.80
A15	CB	19.51
A15	H	7.55
A15	HA	4.35
A15	HB#	1.63
A15	N	120.13
F16	C	177.89
F16	CA	62.35
F16	CB	40.97
F16	CD#	132.40
F16	CE#	131.36
F16	CZ	130.40
F16	H	8.89
F16	HA	3.78
F16	HB2	3.12
F16	HB3	3.36
F16	HD#	6.73
F16	HE#	6.93
F16	HZ	7.37
F16	N	120.41
V17	C	178.57
V17	CA	65.43
V17	CB	31.68
V17	CG1	21.18
V17	CG2	21.29
V17	H	8.53
V17	HA	3.88
V17	HB	2.33
V17	HG1#	1.09
V17	HG2#	1.12
V17	N	113.30
Q18	C	177.02
Q18	CA	57.77
Q18	CB	27.88
Q18	CG	33.80
Q18	H	7.40
Q18	HA	3.93
Q18	HB2	1.95
Q18	HB3	2.06
Q18	HE21	6.80
Q18	HE22	7.40
Q18	HG2	2.39
Q18	HG3	2.48
Q18	N	117.70
Q18	NE2	112.92
F19	C	174.86
F19	CA	58.72
F19	CB	39.72
F19	CD#	132.32
F19	CE#	131.26
F19	CZ	129.09
F19	H	7.68
F19	HA	4.24
F19	HB2	2.66
F19	HB3	3.03
F19	HD#	7.34
F19	HE#	7.27
F19	HZ	7.08
F19	N	116.35
D20	C	176.84
D20	CA	52.19
D20	CB	39.73
D20	H	7.47
D20	HA	4.91
D20	HB2	2.10
D20	HB3	2.73
D20	N	122.61
K21	C	178.70
K21	CA	58.77
K21	CB	32.50
K21	CD	28.93
K21	CE	42.11
K21	CG	25.14
K21	H	8.05
K21	HA	4.02
K21	HB#	1.83
K21	HD#	1.64
K21	HE#	2.97
K21	HG2	1.41
K21	HG3	1.45
K21	N	122.86
E22	C	177.21
E22	CA	56.16
E22	CB	30.08
E22	CG	36.78
E22	H	8.74
E22	HA	4.38
E22	HB2	2.01
E22	HB3	2.24
E22	HG#	2.27
E22	N	115.58
G23	C	176.15
G23	CA	47.41
G23	H	7.95
G23	HA#	3.87
G23	N	110.27
T24	C	176.83
T24	CA	62.26
T24	CB	70.70
T24	CG2	22.20
T24	H	9.29
T24	HA	4.32
T24	HB	4.34
T24	HG2#	1.19
T24	N	113.00
G25	C	173.56
G25	CA	45.43
G25	H	10.74
G25	HA2	3.77
G25	HA3	4.33
G25	N	115.27
K26	C	174.59
K26	CA	54.64
K26	CB	37.07
K26	CD	29.51
K26	CE	42.41
K26	CG	25.21
K26	H	7.72
K26	HA	5.41
K26	HB2	1.50
K26	HB3	1.57
K26	HD2	1.45
K26	HD3	1.58
K26	HE#	2.95
K26	HG2	1.21
K26	HG3	1.31
K26	N	118.66
I27	C	174.94
I27	CA	58.31
I27	CB	41.61
I27	CD1	14.58
I27	CG1	25.36
I27	CG2	17.56
I27	H	8.54
I27	HA	4.55
I27	HB	1.55
I27	HD1#	0.26
I27	HG12	0.74
I27	HG13	1.11
I27	HG2#	-0.05
I27	N	111.47
A28	C	179.72
A28	CA	51.35
A28	CB	19.36
A28	H	8.66
A28	HA	4.80
A28	HB#	1.54
A28	N	124.21
T29	C	177.38
T29	CA	65.76
T29	CB	67.31

T29	CG2	24.15	M36	HB3	2.00	P43	HD2	3.23	L51	H	8.03
T29	H	8.49	M36	HE#	1.76	P43	HD3	3.64	L51	HA	4.07
T29	HA	3.59	M36	HG2	2.46	P43	HG2	1.90	L51	HB2	1.28
T29	HB	4.15	M36	HG3	2.85	P43	HG3	1.99	L51	HB3	2.02
T29	HG2#	1.10	M36	N	117.88	T44	C	175.08	L51	HD1#	0.86
T29	N	115.43	R37	C	181.58	T44	CA	60.94	L51	HD2#	0.74
R30	C	177.01	R37	CA	59.33	T44	CB	70.84	L51	HG	1.92
R30	CA	58.10	R37	CB	30.21	T44	CG2	21.84	L51	N	120.72
R30	CB	29.43	R37	CD	43.60	T44	H	8.91	I52	C	176.97
R30	CD	43.17	R37	CG	29.28	T44	HA	4.36	I52	CA	64.28
R30	CG	27.08	R37	H	8.25	T44	HB	4.68	I52	CB	38.52
R30	H	7.99	R37	HA	4.61	T44	HG2#	1.34	I52	CD1	13.81
R30	HA	4.22	R37	HB2	1.92	T44	N	113.53	I52	CG1	28.21
R30	HB2	1.85	R37	HB3	1.94	E45	C	179.10	I52	CG2	17.72
R30	HB3	1.93	R37	HD2	3.08	E45	CA	59.98	I52	H	8.15
R30	HD#	3.19	R37	HD3	3.24	E45	CB	29.12	I52	HA	3.77
R30	HG2	1.58	R37	HG2	1.82	E45	CG	36.48	I52	HB	1.93
R30	HG3	1.63	R37	HG3	1.89	E45	H	8.82	I52	HD1#	0.77
R30	N	117.50	R37	N	118.64	E45	HA	3.97	I52	HG12	1.11
E31	C	175.97	T38	C	175.43	E45	HB#	2.02	I52	HG13	1.53
E31	CA	55.85	T38	CA	66.28	E45	HG2	2.29	I52	HG2#	0.84
E31	CB	29.85	T38	CB	69.22	E45	HG3	2.36	I52	N	115.48
E31	CG	36.75	T38	CG2	21.32	E45	N	120.76	A53	C	180.14
E31	H	7.78	T38	H	8.14	A46	C	180.47	A53	CA	55.44
E31	HA	4.62	T38	HA	4.19	A46	CA	55.02	A53	CB	18.27
E31	HB2	1.86	T38	HB	4.58	A46	CB	18.30	A53	H	7.75
E31	HB3	2.25	T38	HG2#	1.47	A46	H	8.32	A53	HA	4.08
E31	HG2	2.25	T38	N	117.93	A46	HA	4.06	A53	HB#	1.50
E31	HG3	2.38	L39	C	177.01	A46	HB#	1.36	A53	N	122.86
E31	N	117.85	L39	CA	54.36	A46	N	120.90	E54	C	178.34
L32	C	178.02	L39	CB	42.69	E47	C	180.01	E54	CA	58.97
L32	CA	58.57	L39	CD1	22.95	E47	CA	58.80	E54	CB	29.36
L32	CB	42.09	L39	CD2	26.72	E47	CB	29.87	E54	CG	36.20
L32	CD1	23.32	L39	CG	26.78	E47	CG	37.14	E54	H	8.06
L32	CD2	26.41	L39	H	7.26	E47	H	7.73	E54	HA	4.08
L32	CG	27.04	L39	HA	4.57	E47	HA	4.01	E54	HB#	2.07
L32	H	7.54	L39	HB2	1.75	E47	HB2	1.88	E54	HG2	2.26
L32	HA	3.66	L39	HB3	1.88	E47	HB3	2.28	E54	HG3	2.35
L32	HB2	1.37	L39	HD1#	1.15	E47	HG2	2.24	E54	N	117.67
L32	HB3	1.83	L39	HD2#	0.97	E47	HG3	2.35	A55	C	180.86
L32	HD1#	0.98	L39	HG	1.72	E47	N	118.72	A55	CA	54.11
L32	HD2#	0.85	L39	N	120.63	L48	C	178.27	A55	CB	18.73
L32	HG	1.47	G40	C	174.41	L48	CA	58.03	A55	H	8.04
L32	N	120.01	G40	CA	45.99	L48	CB	41.89	A55	HA	4.28
G33	C	175.15	G40	H	7.77	L48	CD1	24.57	A55	HB#	1.39
G33	CA	48.16	G40	HA2	3.80	L48	CD2	24.75	A55	N	122.22
G33	H	8.95	G40	HA3	4.24	L48	CG	27.17	E56	C	177.59
G33	HA2	3.45	G40	N	106.89	L48	H	8.43	E56	CA	58.98
G33	HA3	3.78	Q41	C	173.88	L48	HA	3.92	E56	CB	29.41
G33	N	106.13	Q41	CA	53.58	L48	HB#	1.65	E56	CG	36.24
T34	C	176.90	Q41	CB	30.22	L48	HD1#	0.80	E56	H	8.62
T34	CA	66.08	Q41	CG	33.41	L48	HD2#	0.80	E56	HA	3.95
T34	CB	68.43	Q41	H	7.93	L48	HG	1.60	E56	HB2	2.04
T34	CG2	22.75	Q41	HA	4.42	L48	N	119.76	E56	HB3	2.11
T34	H	7.77	Q41	HB2	1.52	Q49	C	178.83	E56	HG#	2.29
T34	HA	3.82	Q41	HB3	2.09	Q49	CA	58.88	E56	N	119.00
T34	HB	4.14	Q41	HE21	6.96	Q49	CB	27.93	N57	C	176.44
T34	HG2#	1.39	Q41	HE22	7.65	Q49	CG	33.87	N57	CA	54.83
T34	N	118.34	Q41	HG2	1.98	Q49	H	8.04	N57	CB	39.22
L35	C	178.61	Q41	HG3	2.17	Q49	HA	3.88	N57	H	8.10
L35	CA	58.39	Q41	N	117.91	Q49	HB2	2.10	N57	HA	4.63
L35	CB	40.18	Q41	NE2	113.59	Q49	HB3	2.16	N57	HB2	2.76
L35	CD1	22.72	N42	C	171.91	Q49	HE21	6.82	N57	HB3	2.80
L35	CD2	27.16	N42	CA	51.11	Q49	HE22	7.54	N57	HD21	6.95
L35	CG	27.12	N42	CB	39.52	Q49	HG#	2.47	N57	HD22	7.68
L35	H	7.69	N42	H	8.69	Q49	N	116.65	N57	N	116.96
L35	HA	3.66	N42	HA	5.17	Q49	NE2	112.29	N57	ND2	113.43
L35	HB2	0.32	N42	HB2	2.50	D50	C	178.76	N58	C	175.62
L35	HB3	1.42	N42	HB3	2.73	D50	CA	57.29	N58	CA	54.08
L35	HD1#	0.53	N42	HD21	6.72	D50	CB	40.56	N58	CB	39.89
L35	HD2#	-0.11	N42	HD22	7.53	D50	H	7.56	N58	H	8.52
L35	HG	1.05	N42	N	117.12	D50	HA	4.48	N58	HA	4.84
L35	N	124.86	N42	ND2	112.57	D50	HB2	2.67	N58	HB2	2.80
M36	C	178.65	P43	C	177.61	D50	HB3	2.72	N58	HB3	2.83
M36	CA	61.28	P43	CA	62.43	D50	N	119.26	N58	HD21	6.95
M36	CB	32.89	P43	CB	31.57	L51	C	179.88	N58	HD22	7.73
M36	CE	16.74	P43	CD	50.11	L51	CA	57.79	N58	N	115.77
M36	CG	33.53	P43	CG	27.21	L51	CB	42.52	N58	ND2	113.94
M36	H	8.82	P43	HA	4.71	L51	CD1	22.92	N59	C	175.77
M36	HA	3.74	P43	HB2	1.94	L51	CD2	25.10	N59	CA	53.24
M36	HB2	1.89	P43	HB3	2.07	L51	CG	26.51	N59	CB	39.68

N59	H	8.17	T66	H	8.10	K74	H	7.36	T81	N	115.71
N59	HA	4.96	T66	HA	3.60	K74	HA	4.02	E82	C	177.70
N59	HB2	2.81	T66	HB	4.18	K74	HB2	1.87	E82	CA	58.67
N59	HB3	3.22	T66	HG2#	1.21	K74	HB3	1.91	E82	CB	29.55
N59	HD21	6.90	T66	N	117.33	K74	HD#	1.61	E82	CG	36.40
N59	HD22	7.70	E67	C	180.20	K74	HE#	2.91	E82	H	8.38
N59	N	117.72	E67	CA	58.94	K74	HG2	1.39	E82	HA	4.22
N59	ND2	112.90	E67	CB	29.77	K74	HG3	1.50	E82	HB2	2.10
N60	C	175.56	E67	CG	36.89	K74	N	116.99	E82	HB3	2.12
N60	CA	54.15	E67	H	8.74	Q75	C	177.23	E82	HG2	2.31
N60	CB	38.19	E67	HA	3.97	Q75	CA	56.82	E82	HG3	2.34
N60	H	8.70	E67	HB2	1.90	Q75	CB	28.46	E82	N	122.44
N60	HA	4.58	E67	HB3	2.36	Q75	CG	33.38	E83	C	178.74
N60	HB2	2.74	E67	HG2	2.26	Q75	H	7.88	E83	CA	59.31
N60	HB3	3.03	E67	HG3	2.47	Q75	HA	4.02	E83	CB	29.51
N60	HD21	6.86	E67	N	123.54	Q75	HB#	2.04	E83	CG	36.33
N60	HD22	7.53	F68	C	176.37	Q75	HE21	6.80	E83	H	8.21
N60	N	116.61	F68	CA	61.56	Q75	HE22	7.36	E83	HA	4.04
N60	ND2	112.56	F68	CB	39.14	Q75	HG#	2.20	E83	HB#	2.08
G61	C	174.47	F68	CD#	131.25	Q75	N	118.20	E83	HG2	2.31
G61	CA	46.62	F68	CE#	131.89	Q75	NE2	110.02	E83	HG3	2.35
G61	H	8.39	F68	H	8.67	M76	C	176.25	E83	N	119.70
G61	HA2	3.69	F68	HA	4.08	M76	CA	56.47	E84	C	179.92
G61	HA3	4.12	F68	HB#	3.16	M76	CB	33.25	E84	CA	59.59
G61	N	105.08	F68	HD#	6.94	M76	CE	16.93	E84	CB	29.56
Q62	C	174.45	F68	HE#	7.22	M76	CG	32.09	E84	CG	36.67
Q62	CA	54.23	F68	N	120.98	M76	H	7.81	E84	H	8.24
Q62	CB	33.55	C69	C	176.52	M76	HA	4.25	E84	HA	4.10
Q62	CG	33.80	C69	CA	65.05	M76	HB2	1.99	E84	HB#	2.18
Q62	H	7.51	C69	CB	26.48	M76	HB3	2.19	E84	HG2	2.39
Q62	HA	5.34	C69	H	8.31	M76	HE#	2.08	E84	HG3	2.47
Q62	HB2	1.90	C69	HA	3.34	M76	HG2	2.53	E84	N	118.99
Q62	HB3	1.95	C69	HB2	2.33	M76	HG3	2.66	M85	C	178.52
Q62	HE21	6.75	C69	HB3	2.77	M76	N	116.89	M85	CA	58.66
Q62	HE22	7.33	C69	N	117.70	R77	C	176.57	M85	CB	32.78
Q62	HG2	2.17	G70	C	176.46	R77	CA	56.74	M85	CE	17.49
Q62	HG3	2.30	G70	CA	47.07	R77	CB	30.85	M85	CG	33.16
Q62	N	118.88	G70	H	7.70	R77	CD	43.53	M85	H	8.26
Q62	NE2	111.34	G70	HA2	3.72	R77	CG	27.06	M85	HA	4.54
L63	C	175.94	G70	HA3	3.79	R77	H	7.72	M85	HB2	2.24
L63	CA	53.72	G70	N	106.49	R77	HA	4.27	M85	HB3	2.63
L63	CB	46.18	I71	C	178.17	R77	HB#	1.87	M85	HE#	2.01
L63	CD1	25.72	I71	CA	65.21	R77	HD#	3.20	M85	HG2	2.63
L63	CD2	27.59	I71	CB	38.25	R77	HG#	1.67	M85	HG3	2.85
L63	CG	26.10	I71	CD1	14.92	R77	N	120.95	M85	N	120.23
L63	H	9.05	I71	CG1	29.58	E78	C	176.94	R86	C	179.77
L63	HA	4.98	I71	CG2	17.29	E78	CA	56.69	R86	CA	59.53
L63	HB#	1.61	I71	H	7.53	E78	CB	30.27	R86	CB	29.80
L63	HD1#	0.84	I71	HA	3.57	E78	CG	36.30	R86	CD	42.97
L63	HD2#	0.85	I71	HB	1.81	E78	H	8.48	R86	CG	27.23
L63	HG	1.63	I71	HD1#	0.82	E78	HA	4.35	R86	H	8.49
L63	N	120.89	I71	HG12	1.06	E78	HB2	1.96	R86	HA	4.08
N64	C	175.76	I71	HG13	1.71	E78	HB3	2.09	R86	HB2	1.75
N64	CA	50.92	I71	HG2#	0.79	E78	HG2	2.27	R86	HB3	2.05
N64	CB	39.26	I71	N	123.89	E78	HG3	2.35	R86	HD2	2.81
N64	H	9.24	M72	C	178.68	E78	N	122.44	R86	HD3	2.86
N64	HA	5.65	M72	CA	56.05	T79	C	174.38	R86	HG2	1.48
N64	HB2	2.77	M72	CB	30.36	T79	CA	62.28	R86	HG3	1.76
N64	HB3	3.49	M72	CE	16.85	T79	CB	70.02	R86	N	120.76
N64	HD21	6.89	M72	CG	32.46	T79	CG2	21.68	E87	C	179.03
N64	HD22	7.44	M72	H	7.67	T79	H	8.23	E87	CA	58.96
N64	N	120.49	M72	HA	3.97	T79	HA	4.30	E87	CB	29.08
N64	ND2	110.71	M72	HB2	1.43	T79	HB	4.21	E87	CG	36.03
F65	C	177.45	M72	HB3	1.52	T79	HG2#	1.20	E87	H	8.20
F65	CA	62.01	M72	HE#	1.52	T79	N	115.62	E87	HA	4.05
F65	CB	38.89	M72	HG2	0.90	D80	C	177.07	E87	HB#	2.13
F65	CD#	131.77	M72	HG3	1.04	D80	CA	54.47	E87	HG#	2.39
F65	CE#	130.63	M72	N	117.82	D80	CB	41.23	E87	N	118.90
F65	CZ	130.25	A73	C	180.02	D80	H	8.52	A88	C	179.45
F65	H	8.65	A73	CA	55.06	D80	HA	4.72	A88	CA	55.19
F65	HA	3.61	A73	CB	17.98	D80	HB2	2.70	A88	CB	17.86
F65	HB2	2.32	A73	H	8.33	D80	HB3	2.82	A88	H	8.07
F65	HB3	2.64	A73	HA	3.98	D80	N	123.80	A88	HA	4.24
F65	HD#	6.60	A73	HB#	1.42	T81	C	175.72	A88	HB#	1.82
F65	HE#	7.09	A73	N	120.48	T81	CA	63.57	A88	N	122.58
F65	HZ	7.51	K74	C	178.33	T81	CB	69.51	F89	C	176.76
F65	N	118.33	K74	CA	58.74	T81	CG2	21.92	F89	CA	62.02
T66	C	177.94	K74	CB	32.46	T81	H	8.34	F89	CB	39.45
T66	CA	66.99	K74	CD	29.53	T81	HA	4.27	F89	CD#	131.79
T66	CB	68.42	K74	CE	42.06	T81	HB	4.36	F89	CE#	131.24
T66	CG2	21.89	K74	CG	25.15	T81	HG2#	1.26	F89	CZ	129.56

F89	H	8.45	G96	CA	47.40	E104	HB3	2.65	N111	CA	56.08
F89	HA	3.09	G96	H	7.68	E104	HG#	2.24	N111	CB	38.60
F89	HB2	3.04	G96	HA2	3.80	E104	N	121.10	N111	H	7.77
F89	HB3	3.15	G96	HA3	3.89	L105	C	178.39	N111	HA	4.36
F89	HD#	6.67	G96	N	109.84	L105	CA	58.40	N111	HB2	2.56
F89	HE#	6.98	D97	C	176.84	L105	CB	42.13	N111	HB3	2.62
F89	HZ	7.09	D97	CA	54.05	L105	CD1	24.27	N111	HD21	6.16
F89	N	118.97	D97	CB	40.44	L105	CD2	26.49	N111	HD22	7.20
K90	C	178.11	D97	H	8.49	L105	CG	27.12	N111	N	120.95
K90	CA	59.09	D97	HA	4.47	L105	H	8.53	N111	ND2	112.96
K90	CB	32.67	D97	HB2	2.57	L105	HA	4.15	L112	C	177.83
K90	CD	29.72	D97	HB3	3.14	L105	HB2	1.51	L112	CA	55.55
K90	CE	42.04	D97	N	121.03	L105	HB3	1.95	L112	CB	42.45
K90	CG	25.56	G98	C	172.51	L105	HD1#	0.95	L112	CD1	22.39
K90	H	7.59	G98	CA	45.00	L105	HD2#	0.94	L112	CD2	25.70
K90	HA	3.85	G98	H	10.41	L105	HG	1.67	L112	CG	26.35
K90	HB#	1.91	G98	HA2	3.42	L105	N	120.76	L112	H	7.94
K90	HD#	1.69	G98	HA3	4.01	R106	C	177.17	L112	HA	4.29
K90	HE#	2.91	G98	N	112.40	R106	CA	60.23	L112	HB2	1.68
K90	HG2	1.51	F99	C	174.68	R106	CB	30.58	L112	HB3	1.92
K90	HG3	1.77	F99	CA	56.16	R106	CD	43.45	L112	HD1#	0.83
K90	N	115.58	F99	CB	44.47	R106	CG	28.17	L112	HD2#	0.83
I91	C	177.44	F99	CD#	132.33	R106	H	8.32	L112	HG	1.80
I91	CA	63.59	F99	CE#	131.47	R106	HA	3.76	L112	N	118.97
I91	CB	37.35	F99	CZ	129.99	R106	HB#	1.90	G113	C	174.69
I91	CD1	12.35	F99	H	8.16	R106	HD2	3.17	G113	CA	45.46
I91	CG1	28.57	F99	HA	5.16	R106	HD3	3.23	G113	H	7.88
I91	CG2	16.91	F99	HB2	2.74	R106	HG#	1.60	G113	HA2	3.80
I91	H	7.35	F99	HB3	2.82	R106	N	117.08	G113	HA3	4.16
I91	HA	3.58	F99	HD#	6.90	F107	C	178.09	G113	N	107.29
I91	HB	2.03	F99	HE#	7.44	F107	CA	61.50	E114	C	176.09
I91	HD1#	0.82	F99	HZ	7.27	F107	CB	39.62	E114	CA	55.65
I91	HG12	1.22	F99	N	117.09	F107	CD#	131.98	E114	CB	30.47
I91	HG13	1.65	I100	C	175.68	F107	CE#	131.16	E114	CG	35.69
I91	HG2#	0.64	I100	CA	60.57	F107	CZ	130.63	E114	H	7.87
I91	N	117.94	I100	CB	38.82	F107	H	7.85	E114	HA	4.31
F92	C	176.82	I100	CD1	16.08	F107	HA	4.06	E114	HB2	1.74
F92	CA	59.94	I100	CG1	26.85	F107	HB#	3.26	E114	HB3	1.97
F92	CB	40.79	I100	CG2	18.07	F107	HD#	7.19	E114	HG2	2.12
F92	CD#	131.80	I100	H	10.27	F107	HE#	7.30	E114	HG3	2.26
F92	CE#	131.25	I100	HA	4.75	F107	HZ	6.94	E114	N	119.71
F92	CZ	130.07	I100	HB	1.94	F107	N	117.28	K115	C	175.69
F92	H	7.25	I100	HD1#	0.34	V108	C	178.13	K115	CA	55.61
F92	HA	4.23	I100	HG12	0.20	V108	CA	66.54	K115	CB	31.52
F92	HB2	2.59	I100	HG13	1.24	V108	CB	31.77	K115	CD	29.01
F92	HB3	2.62	I100	HG2#	0.96	V108	CG1	20.90	K115	CE	42.19
F92	HD#	7.29	I100	N	126.34	V108	CG2	23.48	K115	CG	24.61
F92	HE#	7.39	S101	C	173.60	V108	H	8.05	K115	H	8.63
F92	HZ	7.33	S101	CA	55.90	V108	HA	3.44	K115	HA	4.37
F92	N	117.12	S101	CB	63.61	V108	HB	2.00	K115	HB2	1.71
D93	C	177.05	S101	H	9.53	V108	HG1#	0.32	K115	HB3	1.77
D93	CA	52.15	S101	HA	5.09	V108	HG2#	0.92	K115	HD#	1.64
D93	CB	38.38	S101	HB2	3.99	V108	N	118.83	K115	HE#	2.97
D93	H	7.81	S101	HB3	4.42	M109	C	178.83	K115	HG2	1.33
D93	HA	4.50	S101	N	125.97	M109	CA	58.82	K115	HG3	1.42
D93	HB2	1.40	P102	C	178.36	M109	CB	31.90	K115	N	123.87
D93	HB3	2.28	P102	CA	66.53	M109	CE	17.77	V116	C	175.64
D93	N	116.68	P102	CB	31.45	M109	CG	33.46	V116	CA	60.08
R94	C	178.28	P102	CD	49.94	M109	H	8.15	V116	CB	34.65
R94	CA	58.79	P102	CG	28.52	M109	HA	4.13	V116	CG1	20.23
R94	CB	30.51	P102	HA	4.11	M109	HB2	1.90	V116	CG2	21.76
R94	CD	42.98	P102	HB2	2.02	M109	HB3	2.16	V116	H	7.68
R94	CG	27.00	P102	HB3	2.36	M109	HE#	2.03	V116	HA	4.64
R94	H	7.56	P102	HD2	4.00	M109	HG2	2.70	V116	HB	2.02
R94	HA	3.86	P102	HD3	4.06	M109	HG3	2.73	V116	HG1#	0.88
R94	HB2	1.71	P102	HG2	2.08	M109	N	116.78	V116	HG2#	0.89
R94	HB3	1.84	P102	HG3	2.35	I110	C	180.41	V116	N	119.62
R94	HD2	2.97	A103	C	181.59	I110	CA	64.20	T117	C	175.46
R94	HD3	3.12	A103	CA	55.16	I110	CB	37.36	T117	CA	60.30
R94	HG2	1.61	A103	CB	18.52	I110	CD1	12.98	T117	CB	71.73
R94	HG3	1.70	A103	H	8.00	I110	CG1	29.02	T117	CG2	21.65
R94	N	124.53	A103	HA	4.09	I110	CG2	17.08	T117	H	8.61
D95	C	177.94	A103	HB#	1.41	I110	H	8.22	T117	HA	4.58
D95	CA	52.99	A103	N	117.25	I110	HA	3.95	T117	HB	4.71
D95	CB	39.91	E104	C	178.82	I110	HB	1.86	T117	HG2#	1.28
D95	H	8.24	E104	CA	59.46	I110	HD1#	0.82	T117	N	115.95
D95	HA	4.55	E104	CB	29.27	I110	HG12	1.24	D118	C	178.56
D95	HB2	2.58	E104	CG	38.02	I110	HG13	1.62	D118	CA	58.01
D95	HB3	3.05	E104	H	7.98	I110	HG2#	0.82	D118	CB	39.95
D95	N	114.12	E104	HA	4.05	I110	N	118.50	D118	H	8.89
G96	C	175.26	E104	HB2	2.63	N111	C	176.87	D118	HA	4.26

D118	HB2	2.55	I125	HG2#	0.70	G134	N	113.71	F141	HD#	6.91
D118	HB3	2.74	I125	N	118.00	M135	C	174.96	F141	HE#	6.96
D118	N	121.26	R126	C	179.51	M135	CA	52.45	F141	HZ	7.33
E119	C	179.32	R126	CA	59.74	M135	CB	37.34	F141	N	123.20
E119	CA	60.16	R126	CB	30.20	M135	CE	18.13	V142	C	179.45
E119	CB	28.97	R126	CD	43.36	M135	CG	32.28	V142	CA	66.52
E119	CG	37.03	R126	CG	27.73	M135	H	7.94	V142	CB	31.61
E119	H	8.78	R126	H	8.08	M135	HA	4.88	V142	CG1	22.56
E119	HA	4.09	R126	HA	3.92	M135	HB2	1.67	V142	CG2	21.15
E119	HB2	1.95	R126	HB2	1.84	M135	HB3	1.80	V142	H	8.39
E119	HB3	2.05	R126	HB3	1.91	M135	HE#	1.74	V142	HA	3.21
E119	HG2	2.31	R126	HD#	3.20	M135	HG#	2.14	V142	HB	1.87
E119	HG3	2.42	R126	HG2	1.60	M135	N	115.73	V142	HG1#	0.58
E119	N	119.23	R126	HG3	1.74	I136	C	176.28	V142	HG2#	0.76
E120	C	179.88	R126	N	117.60	I136	CA	58.40	V142	N	119.04
E120	CA	59.24	E127	C	176.57	I136	CB	38.13	W143	C	178.56
E120	CB	30.01	E127	CA	58.70	I136	CD1	11.45	W143	CA	61.99
E120	CG	37.76	E127	CB	30.07	I136	CG1	26.86	W143	CB	28.64
E120	H	7.73	E127	CG	36.64	I136	CG2	17.87	W143	CD1	127.08
E120	HA	4.00	E127	H	7.87	I136	H	9.07	W143	CE3	120.17
E120	HB2	1.92	E127	HA	3.90	I136	HA	5.39	W143	CH2	124.63
E120	HB3	2.35	E127	HB#	2.13	I136	HB	2.34	W143	CZ2	114.64
E120	HG#	2.27	E127	HG2	2.38	I136	HD1#	0.80	W143	CZ3	121.81
E120	N	120.56	E127	HG3	2.50	I136	HG12	1.32	W143	H	7.63
I121	C	177.37	E127	N	115.80	I136	HG13	1.36	W143	HA	4.04
I121	CA	63.42	A128	C	175.81	I136	HG2#	1.28	W143	HB2	3.34
I121	CB	36.17	A128	CA	51.75	I136	N	125.05	W143	HB3	3.71
I121	CD1	11.29	A128	CB	20.72	N137	C	175.06	W143	HD1	7.26
I121	CG1	28.03	A128	H	7.13	N137	CA	51.16	W143	HE1	10.10
I121	CG2	17.50	A128	HA	4.15	N137	CB	38.08	W143	HE3	7.48
I121	H	7.96	A128	HB#	1.50	N137	H	9.41	W143	HH2	7.13
I121	HA	3.81	A128	N	118.03	N137	HA	5.20	W143	HZ2	7.45
I121	HB	2.25	D129	C	177.58	N137	HB#	3.33	W143	HZ3	7.01
I121	HD1#	0.77	D129	CA	53.01	N137	HD21	6.72	W143	N	120.78
I121	HG12	1.43	D129	CB	39.55	N137	HD22	7.23	W143	NE1	129.22
I121	HG13	1.49	D129	H	7.47	N137	N	129.19	M144	C	179.02
I121	HG2#	0.97	D129	HA	4.54	N137	ND2	107.85	M144	CA	59.60
I121	N	121.69	D129	HB2	2.42	Y138	C	175.89	M144	CB	34.07
D122	C	179.31	D129	HB3	2.69	Y138	CA	62.91	M144	CE	16.95
D122	CA	57.72	D129	N	115.35	Y138	CB	37.77	M144	CG	31.53
D122	CB	40.45	F130	C	177.92	Y138	CD#	132.51	M144	H	8.70
D122	H	8.05	F130	CA	59.55	Y138	CE#	117.99	M144	HA	3.71
D122	HA	4.33	F130	CB	38.97	Y138	H	8.39	M144	HB#	2.27
D122	HB2	2.63	F130	CD#	131.05	Y138	HA	3.55	M144	HE#	1.91
D122	HB3	2.78	F130	CE#	131.29	Y138	HB2	2.01	M144	HG2	2.28
D122	N	119.27	F130	CZ	129.57	Y138	HB3	2.36	M144	HG3	2.63
E123	C	178.04	F130	H	8.22	Y138	HD#	6.43	M144	N	120.70
E123	CA	59.18	F130	HA	4.32	Y138	HE#	6.52	I145	C	177.63
E123	CB	29.54	F130	HB2	3.02	Y138	N	118.00	I145	CA	61.75
E123	CG	36.08	F130	HB3	3.17	E139	C	180.50	I145	CB	37.28
E123	H	7.86	F130	HD#	7.08	E139	CA	60.55	I145	CD1	12.36
E123	HA	4.05	F130	HE#	6.81	E139	CB	29.12	I145	CG1	26.75
E123	HB#	2.08	F130	HZ	6.07	E139	CG	37.50	I145	CG2	18.03
E123	HG2	2.30	F130	N	126.60	E139	H	8.02	I145	H	8.22
E123	HG3	2.35	D131	C	178.37	E139	HA	3.66	I145	HA	3.85
E123	N	119.54	D131	CA	53.36	E139	HB2	1.98	I145	HB	1.53
M124	C	179.18	D131	CB	39.83	E139	HB3	2.12	I145	HD1#	0.46
M124	CA	59.35	D131	H	8.20	E139	HG2	2.31	I145	HG12	0.79
M124	CB	33.57	D131	HA	4.62	E139	HG3	2.39	I145	HG13	0.84
M124	CE	16.90	D131	HB2	2.65	E139	N	118.06	I145	HG2#	0.48
M124	CG	32.18	D131	HB3	3.08	E140	C	178.35	I145	N	115.18
M124	H	8.04	D131	N	114.92	E140	CA	58.77	S146	C	174.27
M124	HA	4.10	G132	C	175.20	E140	CB	29.77	S146	CA	59.69
M124	HB2	2.10	G132	CA	47.55	E140	CG	37.15	S146	CB	63.70
M124	HB3	2.34	G132	H	7.52	E140	H	8.62	S146	H	7.53
M124	HE#	2.07	G132	HA2	3.78	E140	HA	4.12	S146	HA	4.29
M124	HG2	2.65	G132	HA3	3.88	E140	HB2	2.29	S146	HB#	3.80
M124	HG3	2.76	G132	N	108.57	E140	HB3	2.47	S146	N	116.10
M124	N	119.92	D133	C	177.40	E140	HG2	2.29	Q147	C	175.13
I125	C	176.84	D133	CA	53.65	E140	HG3	2.76	Q147	CA	55.36
I125	CA	64.40	D133	CB	40.13	E140	N	121.49	Q147	CB	28.66
I125	CB	37.05	D133	H	8.28	F141	C	176.25	Q147	CG	32.75
I125	CD1	12.53	D133	HA	4.43	F141	CA	61.40	Q147	H	7.34
I125	CG1	28.66	D133	HB2	2.39	F141	CB	39.50	Q147	HA	3.96
I125	CG2	15.97	D133	HB3	2.86	F141	CD#	132.19	Q147	HB2	1.63
I125	H	8.00	D133	N	120.77	F141	CE#	129.61	Q147	HB3	1.91
I125	HA	3.55	G134	C	173.00	F141	CZ	129.71	Q147	HE21	6.26
I125	HB	2.02	G134	CA	45.64	F141	H	8.93	Q147	HE22	6.70
I125	HD1#	0.77	G134	H	10.54	F141	HA	4.06	Q147	HG2	1.59
I125	HG12	1.12	G134	HA2	3.34	F141	HB2	3.23	Q147	HG3	1.80
I125	HG13	1.68	G134	HA3	3.95	F141	HB3	3.56	Q147	N	119.68

Q147	NE2	113.55	K148	CD	28.88	K148	HA	3.99	K148	HE#	2.80
K148	C	181.29	K148	CE	42.05	K148	HB2	1.57	K148	HG#	1.28
K148	CA	57.63	K148	CG	24.56	K148	HB3	1.72	K148	N	126.33
K148	CB	33.31	K148	H	7.41	K148	HD#	1.53			

C.2 Hydrogen bonds restraints used in structure calculations apoN androcam

File Name : "hbonds-Ca_a.tbl"

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assign ( residue   6 and name O  ) ( residue   10  and name HN ) 1.80  0.00  0.50
assign ( residue   6 and name O  ) ( residue   10  and name N  ) 2.80  0.00  0.50
assign ( residue   7 and name O  ) ( residue   11  and name HN ) 1.80  0.00  0.50
assign ( residue   7 and name O  ) ( residue   11  and name N  ) 2.80  0.00  0.50
assign ( residue   8 and name O  ) ( residue   12  and name HN ) 1.80  0.00  0.50
assign ( residue   8 and name O  ) ( residue   12  and name N  ) 2.80  0.00  0.50
assign ( residue   9 and name O  ) ( residue   13  and name HN ) 1.80  0.00  0.50
assign ( residue   9 and name O  ) ( residue   13  and name N  ) 2.80  0.00  0.50
assign ( residue  10 and name O  ) ( residue   14  and name HN ) 1.80  0.00  0.50
assign ( residue  10 and name O  ) ( residue   14  and name N  ) 2.80  0.00  0.50
assign ( residue  11 and name O  ) ( residue   15  and name HN ) 1.80  0.00  0.50
assign ( residue  11 and name O  ) ( residue   15  and name N  ) 2.80  0.00  0.50
assign ( residue  12 and name O  ) ( residue   16  and name HN ) 1.80  0.00  0.50
assign ( residue  12 and name O  ) ( residue   16  and name N  ) 2.80  0.00  0.50
assign ( residue  13 and name O  ) ( residue   17  and name HN ) 1.80  0.00  0.50
assign ( residue  13 and name O  ) ( residue   17  and name N  ) 2.80  0.00  0.50
assign ( residue  14 and name O  ) ( residue   18  and name HN ) 1.80  0.00  0.50
assign ( residue  14 and name O  ) ( residue   18  and name N  ) 2.80  0.00  0.50
assign ( residue  32 and name O  ) ( residue   36  and name HN ) 1.80  0.00  0.50
assign ( residue  32 and name O  ) ( residue   36  and name N  ) 2.80  0.00  0.50
assign ( residue  33 and name O  ) ( residue   37  and name HN ) 1.80  0.00  0.50
assign ( residue  33 and name O  ) ( residue   37  and name N  ) 2.80  0.00  0.50
assign ( residue  45 and name O  ) ( residue   49  and name HN ) 1.80  0.00  0.50
assign ( residue  45 and name O  ) ( residue   49  and name N  ) 2.80  0.00  0.50
assign ( residue  46 and name O  ) ( residue   50  and name HN ) 1.80  0.00  0.50
assign ( residue  46 and name O  ) ( residue   50  and name N  ) 2.80  0.00  0.50
assign ( residue  47 and name O  ) ( residue   51  and name HN ) 1.80  0.00  0.50
assign ( residue  47 and name O  ) ( residue   51  and name N  ) 2.80  0.00  0.50
assign ( residue  48 and name O  ) ( residue   52  and name HN ) 1.80  0.00  0.50
assign ( residue  48 and name O  ) ( residue   52  and name N  ) 2.80  0.00  0.50
assign ( residue  49 and name O  ) ( residue   53  and name HN ) 1.80  0.00  0.50
assign ( residue  49 and name O  ) ( residue   53  and name N  ) 2.80  0.00  0.50
assign ( residue  50 and name O  ) ( residue   54  and name HN ) 1.80  0.00  0.50
assign ( residue  50 and name O  ) ( residue   54  and name N  ) 2.80  0.00  0.50
assign ( residue  51 and name O  ) ( residue   55  and name HN ) 1.80  0.00  0.50
assign ( residue  51 and name O  ) ( residue   55  and name N  ) 2.80  0.00  0.50
assign ( residue  52 and name O  ) ( residue   56  and name HN ) 1.80  0.00  0.50
assign ( residue  52 and name O  ) ( residue   56  and name N  ) 2.80  0.00  0.50
assign ( residue  65 and name O  ) ( residue   69  and name HN ) 1.80  0.00  0.50
assign ( residue  65 and name O  ) ( residue   69  and name N  ) 2.80  0.00  0.50
assign ( residue  66 and name O  ) ( residue   70  and name HN ) 1.80  0.00  0.50
assign ( residue  66 and name O  ) ( residue   70  and name N  ) 2.80  0.00  0.50
assign ( residue  67 and name O  ) ( residue   71  and name HN ) 1.80  0.00  0.50
assign ( residue  67 and name O  ) ( residue   71  and name N  ) 2.80  0.00  0.50
assign ( residue  68 and name O  ) ( residue   72  and name HN ) 1.80  0.00  0.50
assign ( residue  68 and name O  ) ( residue   72  and name N  ) 2.80  0.00  0.50
assign ( residue  69 and name O  ) ( residue   73  and name HN ) 1.80  0.00  0.50
assign ( residue  69 and name O  ) ( residue   73  and name N  ) 2.80  0.00  0.50
assign ( residue  70 and name O  ) ( residue   74  and name HN ) 1.80  0.00  0.50
assign ( residue  70 and name O  ) ( residue   74  and name N  ) 2.80  0.00  0.50
assign ( residue  71 and name O  ) ( residue   75  and name HN ) 1.80  0.00  0.50
assign ( residue  71 and name O  ) ( residue   75  and name N  ) 2.80  0.00  0.50
assign ( residue  83 and name O  ) ( residue   87  and name HN ) 1.80  0.00  0.50
assign ( residue  83 and name O  ) ( residue   87  and name N  ) 2.80  0.00  0.50
assign ( residue  84 and name O  ) ( residue   88  and name HN ) 1.80  0.00  0.50
assign ( residue  84 and name O  ) ( residue   88  and name N  ) 2.80  0.00  0.50
assign ( residue  85 and name O  ) ( residue   89  and name HN ) 1.80  0.00  0.50
assign ( residue  85 and name O  ) ( residue   89  and name N  ) 2.80  0.00  0.50
assign ( residue  86 and name O  ) ( residue   90  and name HN ) 1.80  0.00  0.50
assign ( residue  86 and name O  ) ( residue   90  and name N  ) 2.80  0.00  0.50
assign ( residue  87 and name O  ) ( residue   91  and name HN ) 1.80  0.00  0.50
assign ( residue  87 and name O  ) ( residue   91  and name N  ) 2.80  0.00  0.50
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assign ( residue   88 and name O  ) ( residue   92  and name HN )  1.80  0.00  0.50
assign ( residue   88 and name O  ) ( residue   92  and name N  )  2.80  0.00  0.50
assign ( residue   89 and name O  ) ( residue   93  and name HN )  1.80  0.00  0.50
assign ( residue   89 and name O  ) ( residue   93  and name N  )  2.80  0.00  0.50
assign ( residue   90 and name O  ) ( residue   94  and name HN )  1.80  0.00  0.50
assign ( residue   90 and name O  ) ( residue   94  and name N  )  2.80  0.00  0.50
assign ( residue   103 and name O  ) ( residue   107  and name HN )  1.80  0.00  0.50
assign ( residue   103 and name O  ) ( residue   107  and name N  )  2.80  0.00  0.50
assign ( residue   104 and name O  ) ( residue   108  and name HN )  1.80  0.00  0.50
assign ( residue   104 and name O  ) ( residue   108  and name N  )  2.80  0.00  0.50
assign ( residue   105 and name O  ) ( residue   109  and name HN )  1.80  0.00  0.50
assign ( residue   105 and name O  ) ( residue   109  and name N  )  2.80  0.00  0.50
assign ( residue   106 and name O  ) ( residue   110  and name HN )  1.80  0.00  0.50
assign ( residue   106 and name O  ) ( residue   110  and name N  )  2.80  0.00  0.50
assign ( residue   107 and name O  ) ( residue   111  and name HN )  1.80  0.00  0.50
assign ( residue   107 and name O  ) ( residue   111  and name N  )  2.80  0.00  0.50
assign ( residue   118 and name O  ) ( residue   122  and name HN )  1.80  0.00  0.50
assign ( residue   118 and name O  ) ( residue   122  and name N  )  2.80  0.00  0.50
assign ( residue   119 and name O  ) ( residue   123  and name HN )  1.80  0.00  0.50
assign ( residue   119 and name O  ) ( residue   123  and name N  )  2.80  0.00  0.50
assign ( residue   120 and name O  ) ( residue   124  and name HN )  1.80  0.00  0.50
assign ( residue   120 and name O  ) ( residue   124  and name N  )  2.80  0.00  0.50
assign ( residue   121 and name O  ) ( residue   125  and name HN )  1.80  0.00  0.50
assign ( residue   121 and name O  ) ( residue   125  and name N  )  2.80  0.00  0.50
assign ( residue   122 and name O  ) ( residue   126  and name HN )  1.80  0.00  0.50
assign ( residue   122 and name O  ) ( residue   126  and name N  )  2.80  0.00  0.50
assign ( residue   123 and name O  ) ( residue   127  and name HN )  1.80  0.00  0.50
assign ( residue   123 and name O  ) ( residue   127  and name N  )  2.80  0.00  0.50
assign ( residue   138 and name O  ) ( residue   142  and name HN )  1.80  0.00  0.50
assign ( residue   138 and name O  ) ( residue   142  and name N  )  2.80  0.00  0.50
assign ( residue   139 and name O  ) ( residue   143  and name HN )  1.80  0.00  0.50
assign ( residue   139 and name O  ) ( residue   143  and name N  )  2.80  0.00  0.50
assign ( residue   140 and name O  ) ( residue   144  and name HN )  1.80  0.00  0.50
assign ( residue   140 and name O  ) ( residue   144  and name N  )  2.80  0.00  0.50
assign ( residue   141 and name O  ) ( residue   145  and name HN )  1.80  0.00  0.50
assign ( residue   141 and name O  ) ( residue   145  and name N  )  2.80  0.00  0.50

! BETA sheet hydrogen bonding in N lobe

assign ( residue   27 and name O  ) ( residue   63  and name HN )  1.80  0.00  0.40
assign ( residue   27 and name O  ) ( residue   63  and name N  )  2.80  0.00  0.40
assign ( residue   63 and name O  ) ( residue   27  and name HN )  1.80  0.00  0.40
assign ( residue   63 and name O  ) ( residue   27  and name N  )  2.80  0.00  0.40
assign ( residue   61 and name O  ) ( residue   29  and name HN )  1.80  0.00  0.40
assign ( residue   61 and name O  ) ( residue   29  and name N  )  2.80  0.00  0.40

! BETA sheet hydrogen bonding in C lobe

assign ( residue   100 and name O  ) ( residue   136  and name HN )  1.80  0.00  0.40
assign ( residue   100 and name O  ) ( residue   136  and name N  )  2.80  0.00  0.40
assign ( residue   136 and name O  ) ( residue   100  and name HN )  1.80  0.00  0.40
assign ( residue   136 and name O  ) ( residue   100  and name N  )  2.80  0.00  0.40

! Ca+2 tying in the C lobe

assign ( residue   149  ) ( residue   93  and name OD1 )  2.27  0.00  0.00
assign ( residue   149  ) ( residue   95  and name OD1  )  2.45  0.00  0.00
assign ( residue   149  ) ( residue   97  and name OD1  )  2.36  0.00  0.00
assign ( residue   149  ) ( residue   99  and name O  )  2.29  0.00  0.00
assign ( residue   149  ) ( residue   104  and name OE1  )  2.46  0.00  0.00
assign ( residue   149  ) ( residue   104  and name OE2  )  2.52  0.00  0.00
assign ( residue   150  ) ( residue   129  and name OD1 )  2.27  0.00  0.00
assign ( residue   150  ) ( residue   131  and name OD1  )  2.45  0.00  0.00
assign ( residue   150  ) ( residue   133  and name OD1  )  2.36  0.00  0.00
assign ( residue   150  ) ( residue   135  and name O  )  2.29  0.00  0.00
assign ( residue   150  ) ( residue   140  and name OE1  )  2.46  0.00  0.00
assign ( residue   150  ) ( residue   140  and name OE2  )  2.52  0.00  0.00
```

C.3 χ_1, χ_2, ψ dihedral restraints for apoN androcam

File name : "chi1-psi.tbl"

```
!   ACaM side chain "chi1" angles  for HBs derived on 2010-02-02 from HNHB and HNCOHB
data
!   chi1 = 180 deg for a HB is trans to N
!   chi1 = -60 or +60 deg for a HB gauche to N
!      S2
assign (resid  2 and name  N) (resid  2 and name CA)
      (resid  2 and name CB) (resid  2 and name OG) 1.00    180.00     30.00 2
!      E3
assign (resid  3 and name  N) (resid  3 and name CA)
      (resid  3 and name CB) (resid  3 and name HB3) 1.00    180.00     30.00 2
!      E7
assign (resid  7 and name  N) (resid  7 and name CA)
      (resid  7 and name CB) (resid  7 and name HB2) 1.00    180.00     30.00 2
!      Q8
assign (resid  8 and name  N) (resid  8 and name CA)
      (resid  8 and name CB) (resid  8 and name HB2) 1.00    180.00     30.00 2
!      E11
assign (resid  11 and name  N) (resid  11 and name CA)
      (resid  11 and name CB) (resid  11 and name CG) 1.00    180.00     30.00 2
!      F12
assign (resid  12 and name  N) (resid  12 and name CA)
      (resid  12 and name CB) (resid  12 and name HB2) 1.00   180.00     30.00 2
!      D14
assign (resid  14 and name  N) (resid  14 and name CA)
      (resid  14 and name CB) (resid  14 and name HB2) 1.00    180.00     30.00 2
!      Q18
assign (resid  18 and name  N) (resid  18 and name CA)
      (resid  18 and name CB) (resid  18 and name HB2) 1.00    180.00     30.00 2
!      F19
assign (resid  19 and name  N) (resid  19 and name CA)
      (resid  19 and name CB) (resid  19 and name HB3) 1.00    180.00     30.00 2
!      D20
assign (resid  20 and name  N) (resid  20 and name CA)
      (resid  20 and name CB) (resid  20 and name CG) 1.00    180.00     30.00 2
!      E22
assign (resid  22 and name  N) (resid  22 and name CA)
      (resid  22 and name CB) (resid  22 and name HB3) 1.00    180.00     30.00 2
!      E31
assign (resid  31 and name  N) (resid  31 and name CA)
      (resid  31 and name CB) (resid  31 and name CG) 1.00    180.00     30.00 2
!      L35
assign (resid  35 and name  N) (resid  35 and name CA)
      (resid  35 and name CB) (resid  35 and name HB3) 1.00    180.00     30.00 2
!      M36
assign (resid  36 and name  N) (resid  36 and name CA)
      (resid  36 and name CB) (resid  36 and name HB2) 1.00    180.00     30.00 2
!      R37
assign (resid  37 and name  N) (resid  37 and name CA)
      (resid  37 and name CB) (resid  37 and name HB3) 1.00    180.00     30.00 2
!      L39
assign (resid  39 and name  N) (resid  39 and name CA)
      (resid  39 and name CB) (resid  39 and name HB3) 1.00    180.00     30.00 2
!      Q41
assign (resid  41 and name  N) (resid  41 and name CA)
      (resid  41 and name CB) (resid  41 and name HB3) 1.00    180.00     30.00 2
!      N42
assign (resid  42 and name  N) (resid  42 and name CA)
      (resid  42 and name CB) (resid  42 and name HB3) 1.00    180.00     30.00 2
!      E47
assign (resid  47 and name  N) (resid  47 and name CA)
      (resid  47 and name CB) (resid  47 and name HB2) 1.00    180.00     30.00 2
!      Q49
assign (resid  49 and name  N) (resid  49 and name CA)
      (resid  49 and name CB) (resid  49 and name HB2) 1.00    180.00     30.00 2
!      D50
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assign (resid  50 and name  N) (resid  50 and name CA)
      (resid  50 and name CB) (resid  50 and name HB2) 1.00    180.00     30.00 2
!      L51
assign (resid  51 and name  N) (resid  51 and name CA)
      (resid  51 and name CB) (resid  51 and name HB2) 1.00    180.00     30.00 2
!      E54
assign (resid  54 and name  N) (resid  54 and name CA)
      (resid  54 and name CB) (resid  54 and name CG) 1.00    180.00     30.00 2
!      N57
assign (resid  57 and name  C) (resid  57 and name CA)
      (resid  57 and name CB) (resid  57 and name HB2) 1.00    180.00     30.00 2
!      N59
assign (resid  59 and name  N) (resid  59 and name CA)
      (resid  59 and name CB) (resid  59 and name HB3) 1.00    180.00     30.00 2
!      N60
assign (resid  60 and name  N) (resid  60 and name CA)
      (resid  60 and name CB) (resid  60 and name HB3) 1.00    180.00     30.00 2
!      Q62
assign (resid  62 and name  N) (resid  62 and name CA)
      (resid  62 and name CB) (resid  62 and name HB2) 1.00    180.00     30.00 2
!      N64
assign (resid  64 and name  N) (resid  64 and name CA)
      (resid  64 and name CB) (resid  64 and name HB3) 1.00    180.00     30.00 2
!      E67
assign (resid  67 and name  N) (resid  67 and name CA)
      (resid  67 and name CB) (resid  67 and name HB2) 1.00    180.00     30.00 2
!      F68
assign (resid  68 and name  N) (resid  68 and name CA)
      (resid  68 and name CB) (resid  68 and name CG) 1.00    180.00     30.00 2
!      C69
assign (resid  69 and name  N) (resid  69 and name CA)
      (resid  69 and name CB) (resid  69 and name HB2) 1.00    180.00     30.00 2
!      M72
assign (resid  72 and name  N) (resid  72 and name CA)
      (resid  72 and name CB) (resid  72 and name HB2) 1.00    180.00     30.00 2
!      M76
assign (resid  76 and name  N) (resid  76 and name CA)
      (resid  76 and name CB) (resid  76 and name HB3) 1.00    180.00     30.00 2
!      E78
assign (resid  78 and name  N) (resid  78 and name CA)
      (resid  78 and name CB) (resid  78 and name HB3) 1.00    180.00     30.00 2
!      D80
assign (resid  80 and name  N) (resid  80 and name CA)
      (resid  80 and name CB) (resid  80 and name HB3) 1.00    180.00     30.00 2
!      E82
assign (resid  82 and name  N) (resid  82 and name CA)
      (resid  82 and name CB) (resid  82 and name HB3) 1.00    180.00     30.00 2
!      F92
assign (resid  92 and name  N) (resid  92 and name CA)
      (resid  92 and name CB) (resid  92 and name HB3) 1.00    180.00     30.00 2
!      D93
assign (resid  93 and name  N) (resid  93 and name CA)
      (resid  93 and name CB) (resid  93 and name CG) 1.00    180.00     30.00 2
!      R94
assign (resid  94 and name  N) (resid  94 and name CA)
      (resid  94 and name CB) (resid  94 and name HB3) 1.00    180.00     30.00 2
!      D95
assign (resid  95 and name  N) (resid  95 and name CA)
      (resid  95 and name CB) (resid  95 and name HB3) 1.00    180.00     30.00 2
!      D97
assign (resid  97 and name  N) (resid  97 and name CA)
      (resid  97 and name CB) (resid  97 and name HB3) 1.00    180.00     30.00 2
!     F99
assign (resid  99 and name  N) (resid  99 and name CA)
      (resid  99 and name CB) (resid  99 and name HB2) 1.00    180.00     30.00 2
!      E104
assign (resid  104 and name  N) (resid  104 and name CA)
      (resid  104 and name CB) (resid  104 and name HB3) 1.00    180.00     30.00 2
!      L105
assign (resid  105 and name  C) (resid  105 and name CA)
      (resid  105 and name CB) (resid  105 and name HB2) 1.00    180.00     30.00 2
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!      F107
assign (resid  107 and name  N) (resid  107 and name CA)
      (resid  107 and name CB) (resid  107 and name CG) 1.00    180.00     30.00 2
!     M109
assign (resid  109 and name  N) (resid  109 and name CA)
      (resid  109 and name CB) (resid  109 and name HB2) 1.00    180.00     30.00 2
!      L112
assign (resid  112 and name  N) (resid  112 and name CA)
      (resid  112 and name CB) (resid  112 and name HB2) 1.00    180.00     30.00 2
!      E114
assign (resid  114 and name  N) (resid  114 and name CA)
      (resid  114 and name CB) (resid  114 and name HB3) 1.00    180.00     30.00 2
!      K115
assign (resid  115 and name  N) (resid  115 and name CA)
      (resid  115 and name CB) (resid  115 and name HB3) 1.00    180.00     30.00 2
!      D118
assign (resid  118 and name  N) (resid  118 and name CA)
      (resid  118 and name CB) (resid  118 and name HB3) 1.00    180.00     30.00 2
!      E119
assign (resid  119 and name  N) (resid  119 and name CA)
      (resid  119 and name CB) (resid  119 and name HB2) 1.00    180.00     30.00 2
!     E120
assign (resid  120 and name  N) (resid  120 and name CA)
      (resid  120 and name CB) (resid  120 and name HB2) 1.00    180.00     30.00 2
!      D122
assign (resid  122 and name  N) (resid  122 and name CA)
      (resid  122 and name CB) (resid  122 and name HB2) 1.00    180.00     30.00 2
!      M124
assign (resid  124 and name  N) (resid  124 and name CA)
      (resid  124 and name CB) (resid  124 and name HB2) 1.00    180.00     30.00 2
!      R126
assign (resid  126 and name  N) (resid  126 and name CA)
      (resid  126 and name CB) (resid  126 and name HB2) 1.00    180.00     30.00 2
!      D129
assign (resid  129 and name  C) (resid  129 and name CA)
      (resid  129 and name CB) (resid  129 and name HB2) 1.00    180.00     30.00 2
!      F130
assign (resid  130 and name  N) (resid  130 and name CA)
      (resid  130 and name CB) (resid  130 and name HB3) 1.00    180.00     30.00 2
!      D131
assign (resid  131 and name  N) (resid  131 and name CA)
      (resid  131 and name CB) (resid  131 and name HB3) 1.00    180.00     30.00 2
!      D133
assign (resid  133 and name  N) (resid  133 and name CA)
      (resid  133 and name CB) (resid  133 and name HB3) 1.00    180.00     30.00 2
!      M135
assign (resid  135 and name  N) (resid  135 and name CA)
      (resid  135 and name CB) (resid  135 and name HB2) 1.00    180.00     30.00 2
!      Y138
!assign (resid  138 and name  N) (resid  138 and name CA)
!      (resid  138 and name CB) (resid  138 and name HB3) 1.00    180.00     30.00 2
!      E139
assign (resid  139 and name  N) (resid  139 and name CA)
      (resid  139 and name CB) (resid  139 and name HB2) 1.00    180.00     30.00 2
!      E140
assign (resid  140 and name  N) (resid  140 and name CA)
      (resid  140 and name CB) (resid  140 and name HB2) 1.00    180.00     30.00 2
!      F141
assign (resid  141 and name  N) (resid  141 and name CA)
      (resid  141 and name CB) (resid  141 and name CG) 1.00    180.00     30.00 2
!     W143
assign (resid  143 and name  C) (resid  143 and name CA)
      (resid  143 and name CB) (resid  143 and name HB2) 1.00    180.00     30.00 2
!      M144
assign (resid  144 and name  N) (resid  144 and name CA)
      (resid  144 and name CB) (resid  144 and name CG) 1.00    180.00     30.00 2
!      Q147
assign (resid  147 and name  N) (resid  147 and name CA)
      (resid  147 and name CB) (resid  147 and name HB3) 1.00    180.00     30.00 2
!      K148
assign (resid  148 and name  N) (resid  148 and name CA)
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      (resid  148 and name CB) (resid  148 and name HB3) 1.00    180.00     30.00 2

!   ACaM side chain "chi1" angles  for CH3s derived on 2010-01-29 from 3JCN, 3JCCO and
HNHB data for ILE, VAL and THR

!      I9
assign (resid  9 and name  N) (resid  9 and name CA)
      (resid  9 and name CB) (resid  9 and name CG2) 1.00    180.00     30.00 2
!      I71
assign (resid  71 and name  N) (resid  71 and name CA)
      (resid  71 and name CB) (resid  71 and name CG2) 1.00    180.00     30.00 2
!      I91
assign (resid  91 and name  N) (resid  91 and name CA)
      (resid  91 and name CB) (resid  91 and name CG2) 1.00    180.00     30.00 2
!      I100
assign (resid  100 and name  N) (resid  100 and name CA)
      (resid  100 and name CB) (resid  100 and name CG2) 1.00    180.00     30.00 2
!      I110
assign (resid  110 and name  N) (resid  110 and name CA)
      (resid  110 and name CB) (resid  110 and name CG2) 1.00    180.00     30.00 2
!      I121
assign (resid  121 and name  N) (resid  121 and name CA)
      (resid  121 and name CB) (resid  121 and name CG2) 1.00    180.00     30.00 2
!      I125
assign (resid  125 and name  N) (resid  125 and name CA)
      (resid  125 and name CB) (resid  125 and name CG2) 1.00    180.00     30.00 2
!      I136
assign (resid  136 and name  N) (resid  136 and name CA)
      (resid  136 and name CB) (resid  136 and name CG2) 1.00    180.00     30.00 2
!      I145
assign (resid  145 and name  N) (resid  145 and name CA)
      (resid  145 and name CB) (resid  145 and name HB) 1.00    180.00     30.00 2
!      T24
assign (resid  24 and name  N) (resid  24 and name CA)
      (resid  24 and name CB) (resid  24 and name HB) 1.00    180.00     30.00 2
!      T29
assign (resid  29 and name  C) (resid  29 and name CA)
      (resid  29 and name CB) (resid  29 and name CG2) 1.00    180.00     30.00 2
!      T44
assign (resid  44 and name  C) (resid  44 and name CA)
      (resid  44 and name CB) (resid  44 and name CG2) 1.00    180.00     30.00 2
!      T81
assign (resid  81 and name  N) (resid  81 and name CA)
      (resid  81 and name CB) (resid  81 and name HB) 1.00    180.00     30.00 2
!      T117
assign (resid  117 and name  N) (resid  117 and name CA)
      (resid  117 and name CB) (resid  117 and name HB) 1.00    180.00     30.00 2
!      V108
assign (resid  108 and name  N) (resid 108 and name CA)
      (resid  108 and name CB) (resid  108 and name CG1) 1.00    180.00     30.00 2
!      V142
assign (resid  142 and name  N) (resid 142 and name CA)
      (resid  142 and name CB) (resid  142 and name CG2) 1.00    180.00     30.00 2

!   ACaM side chain "chi2" angles  for CH3s derived on 2010-01-29 from LRCC data for ILE
and LEU
!      I9
assign (resid  9 and name  CA) (resid 9 and name CB)
      (resid  9 and name CG1) (resid  9 and name CD1) 1.00    180.00     30.00 2
!      I27
assign (resid  27 and name  CA) (resid 27 and name CB)
      (resid  27 and name CG1) (resid  27 and name CD1) 1.00    180.00     30.00 2
!      I52
assign (resid  52 and name  CA) (resid 52 and name CB)
      (resid  52 and name CG1) (resid  52 and name CD1) 1.00    180.00     30.00 2
!      I71
assign (resid  71 and name  CA) (resid 71 and name CB)
      (resid  71 and name CG1) (resid  71 and name CD1) 1.00    180.00     30.00 2
!      I91
assign (resid  91 and name  CA) (resid 91 and name CB)
      (resid  91 and name CG1) (resid  91 and name CD1) 1.00    180.00     30.00 2
```

```
!      I100
assign (resid  100 and name  CA) (resid 100 and name CB)
      (resid  100 and name CG1) (resid  100 and name CD1) 1.00    180.00     30.00 2
!      I125
assign (resid  125 and name  CA) (resid 125 and name CB)
      (resid  125 and name CG1) (resid  125 and name CD1) 1.00    180.00     30.00 2
!      L32
assign (resid  32 and name  CA) (resid 32 and name CB)
      (resid  32 and name CG) (resid  32 and name CD2) 1.00    180.00     30.00 2
!      L35
assign (resid  35 and name  CA) (resid 35 and name CB)
      (resid  35 and name CG) (resid  35 and name CD2) 1.00    180.00     30.00 2
!      L39
assign (resid  39 and name  CA) (resid 39 and name CB)
      (resid  39 and name CG) (resid  39 and name CD2) 1.00    180.00     30.00 2
!      L51
assign (resid  51 and name  CA) (resid 51 and name CB)
      (resid  51 and name CG) (resid  51 and name CD2) 1.00    180.00     30.00 2
!      L63
assign (resid  63 and name  CA) (resid 63 and name CB)
      (resid  63 and name CG) (resid  63 and name CD2) 1.00    180.00     30.00 2
!      L105
assign (resid  105 and name  CA) (resid 105 and name CB)
      (resid  105 and name CG) (resid  105 and name CD2) 1.00    180.00     30.00 2
!      L112
assign (resid  112 and name  CA) (resid 112 and name CB)
      (resid  112 and name CG) (resid  112 and name CD2) 1.00    180.00     30.00 2

!   ACaM backbone "psi" angles derived on 2010/01/11  from HNHA
!  S2
assign (resid 2 and name N)  (resid 2 and name CA) (resid 2 and name C) (resid 3 and name
N)  1.00  -35.00  20.00  2
!  E6
assign (resid 6 and name N)  (resid 6 and name CA) (resid 6 and name C) (resid 7 and name
N)  1.00  -35.00  20.00  2
!  E7
assign (resid 7 and name N) (resid 7 and name CA) (resid 7 and name C) (resid 8 and name
N)  1.00  -35.00  20.00  2
!  Q8
assign (resid 8 and name N) (resid 8 and name CA) (resid 8 and name C) (resid 9 and name
N)  1.00  -35.00  20.00  2
!  I9
assign (resid 9 and name N) (resid 9 and name CA) (resid 9 and name C) (resid 10 and name
N)  1.00  -35.00  20.00  2
!  A10
assign (resid 10 and name N) (resid 10 and name CA) (resid 10 and name C) (resid 11 and
name N)  1.00  -35.00  20.00  2
!  F12
assign (resid 12 and name N) (resid 12 and name CA) (resid 12 and name C) (resid 13 and
name N)  1.00  -35.00  20.00  2
!  K13
assign (resid 13 and name N) (resid 13 and name CA) (resid 13 and name C) (resid 14 and
name N)  1.00  -35.00  20.00  2
!  D14
assign (resid 14 and name N) (resid 14 and name CA) (resid 14 and name C) (resid 15 and
name N)  1.00  -35.00  20.00  2
!  A15
assign (resid 15 and name N) (resid 15 and name CA) (resid 15 and name C) (resid 16 and
name N)  1.00  -35.00  20.00  2
!  F16
assign (resid 16 and name N) (resid 16 and name CA) (resid 16 and name C) (resid 17 and
name N)  1.00  -35.00  20.00  2
!  V17
assign (resid 17 and name N) (resid 17 and name CA) (resid 17 and name C) (resid 18 and
name N)  1.00  -35.00  20.00  2
!  Q18
assign (resid 18 and name N) (resid 18 and name CA) (resid 18 and name C) (resid 19 and
name N)  1.00  -35.00  20.00  2
!  F19
assign (resid 19 and name N) (resid 19 and name CA) (resid 19 and name C) (resid 20 and
name N)  1.00  135.00  20.00  2
```

```
!  T24
assign (resid 24 and name N) (resid 24 and name CA) (resid 24 and name C) (resid 25 and
name N)  1.00  135.00  40.00  2
!  T29
assign (resid 29 and name N) (resid 29 and name CA) (resid 29 and name C) (resid 30 and
name N)  1.00  -35.00  40.00  2
!  R30
assign (resid 30 and name N) (resid 30 and name CA) (resid 30 and name C) (resid 31 and
name N)  1.00  -35.00  40.00  2
!  T34
assign (resid 34 and name N) (resid 34 and name CA) (resid 34 and name C) (resid 35 and
name N)  1.00  -35.00  20.00  2
!  L35
assign (resid 35 and name N) (resid 35 and name CA) (resid 35 and name C) (resid 36 and
name N)  1.00  -35.00  20.00  2
!  M36
assign (resid 36 and name N) (resid 36 and name CA) (resid 36 and name C) (resid 37 and
name N)  1.00  -35.00  20.00  2
!  R37
assign (resid 37 and name N) (resid 37 and name CA) (resid 37 and name C) (resid 38 and
name N)  1.00  -35.00  20.00  2
!  T38
assign (resid 38 and name N) (resid 38 and name CA) (resid 38 and name C) (resid 39 and
name N)  1.00  -35.00  20.00  2
!  Q41
assign (resid 41 and name N) (resid 41 and name CA) (resid 41 and name C) (resid 42 and
name N)  1.00  135.00  20.00  2
!  N42
assign (resid 42 and name N) (resid 42 and name CA) (resid 42 and name C) (resid 43 and
name N)  1.00  135.00  20.00  2
!  E45
assign (resid 45 and name N) (resid 45 and name CA) (resid 45 and name C) (resid 46 and
name N)  1.00  -35.00  20.00  2
!  A46
assign (resid 46 and name N) (resid 46 and name CA) (resid 46 and name C) (resid 47 and
name N)  1.00  -35.00  20.00  2
!  L48
assign (resid 48 and name N) (resid 48 and name CA) (resid 48 and name C) (resid 49 and
name N)  1.00  -35.00  20.00  2
!  Q49
assign (resid 49 and name N) (resid 49 and name CA) (resid 49 and name C) (resid 50 and
name N)  1.00  -35.00  20.00  2
!  D50
assign (resid 50 and name N) (resid 50 and name CA) (resid 50 and name C) (resid 51 and
name N)  1.00  -35.00  20.00  2
!  L51
assign (resid 51 and name N) (resid 51 and name CA) (resid 51 and name C) (resid 52 and
name N)  1.00  -35.00  20.00  2
!  A53
assign (resid 53 and name N) (resid 53 and name CA) (resid 53 and name C) (resid 54 and
name N)  1.00  -35.00  20.00  2
!  E54
assign (resid 54 and name N) (resid 54 and name CA) (resid 54 and name C) (resid 55 and
name N)  1.00  -35.00  20.00  2
!  A55
assign (resid 55 and name N) (resid 55 and name CA) (resid 55 and name C) (resid 56 and
name N)  1.00  -35.00  20.00  2
!  E56
assign (resid 56 and name N) (resid 56 and name CA) (resid 56 and name C) (resid 57 and
name N)  1.00  -35.00  20.00  2
!  N60
assign (resid 60 and name N) (resid 60 and name CA) (resid 60 and name C) (resid 61 and
name N)  1.00  -35.00  20.00  2
!  Q62
assign (resid 62 and name N) (resid 62 and name CA) (resid 62 and name C) (resid 63 and
name N)  1.00  135.00  20.00  2
!  L63
assign (resid 63 and name N) (resid 63 and name CA) (resid 63 and name C) (resid 64 and
name N)  1.00  135.00  20.00  2
!  N64
```

```
assign (resid 64 and name N) (resid 64 and name CA) (resid 64 and name C) (resid 65 and
name N)  1.00  135.00  20.00  2
!  F65
assign (resid 65 and name N) (resid 65 and name CA) (resid 65 and name C) (resid 66 and
name N)  1.00  -35.00  20.00  2
!  T66
assign (resid 66 and name N) (resid 66 and name CA) (resid 66 and name C) (resid 67 and
name N)  1.00  -35.00  20.00  2
!  E67
assign (resid 67 and name N) (resid 67 and name CA) (resid 67 and name C) (resid 68 and
name N)  1.00  -35.00  20.00  2
!  F68
assign (resid 68 and name N) (resid 68 and name CA) (resid 68 and name C) (resid 69 and
name N)  1.00  -35.00  20.00  2
!  C69
assign (resid 69 and name N) (resid 69 and name CA) (resid 69 and name C) (resid 70 and
name N)  1.00  -35.00  20.00  2
!  M72
assign (resid 72 and name N) (resid 72 and name CA) (resid 72 and name C) (resid 73 and
name N)  1.00  -35.00  20.00  2
!  A73
assign (resid 73 and name N) (resid 73 and name CA) (resid 73 and name C) (resid 74 and
name N)  1.00  -35.00  20.00  2
!  K74
assign (resid 74 and name N) (resid 74 and name CA) (resid 74 and name C) (resid 75 and
name N)  1.00  -35.00  20.00  2
!  Q75
assign (resid 75 and name N) (resid 75 and name CA) (resid 75 and name C) (resid 76 and
name N)  1.00  -35.00  20.00  2
!  R77
assign (resid 77 and name N) (resid 77 and name CA) (resid 77 and name C) (resid 78 and
name N)  1.00  -35.00  20.00  2
!  E83
assign (resid 83 and name N) (resid 83 and name CA) (resid 83 and name C) (resid 84 and
name N)  1.00  -35.00  20.00  2
!  E84
assign (resid 84 and name N) (resid 84 and name CA) (resid 84 and name C) (resid 85 and
name N)  1.00  -35.00  20.00  2
!  M85
assign (resid 85 and name N) (resid 85 and name CA) (resid 85 and name C) (resid 86 and
name N)  1.00  -35.00  20.00  2
!  R86
assign (resid 86 and name N) (resid 86 and name CA) (resid 86 and name C) (resid 87 and
name N)  1.00  -35.00  20.00  2
!  E87
assign (resid 87 and name N) (resid 87 and name CA) (resid 87 and name C) (resid 88 and
name N)  1.00  -35.00  20.00  2
!  F89
assign (resid 89 and name N) (resid 89 and name CA) (resid 89 and name C) (resid 90 and
name N)  1.00  -35.00  20.00  2
!  K90
assign (resid 90 and name N) (resid 90 and name CA) (resid 90 and name C) (resid 91 and
name N)  1.00  -35.00  20.00  2
!  I91
assign (resid 91 and name N) (resid 91 and name CA) (resid 91 and name C) (resid 92 and
name N)  1.00  -35.00  20.00  2
!  R94
assign (resid 94 and name N) (resid 94 and name CA) (resid 94 and name C) (resid 95 and
name N)  1.00  -35.00  40.00  2
!  F99
assign (resid 99 and name N) (resid 99 and name CA) (resid 99 and name C) (resid 100 and
name N)  1.00  135.00  20.00  2
!  I100
assign (resid 100 and name N) (resid 100 and name CA) (resid 100 and name C) (resid 101
and name N)  1.00  135.00  20.00  2
!  A103
assign (resid 103 and name N) (resid 103 and name CA) (resid 103 and name C) (resid 104
and name N)  1.00  -35.00  20.00  2
!  L105
assign (resid 105 and name N) (resid 105 and name CA) (resid 105 and name C) (resid 106
and name N)  1.00  -35.00  20.00  2
```

```
!  R106
assign (resid 106 and name N) (resid 106 and name CA) (resid 106 and name C) (resid 107
and name N)  1.00  -35.00  20.00  2
!  F107
assign (resid 107 and name N) (resid 107 and name CA) (resid 107 and name C) (resid 108
and name N)  1.00  -35.00  20.00  2
!  L112
assign (resid 112 and name N) (resid 112 and name CA) (resid 112 and name C) (resid 113
and name N)  1.00  -35.00  20.00  2
!  V116
assign (resid 116 and name N) (resid 116 and name CA) (resid 116 and name C) (resid 117
and name N)  1.00  135.00  20.00  2
!  T117
assign (resid 117 and name N) (resid 117 and name CA) (resid 117 and name C) (resid 118
and name N)  1.00  135.00  20.00  2
!  D118
assign (resid 118 and name N) (resid 118 and name CA) (resid 118 and name C) (resid 119
and name N)  1.00  -35.00  20.00  2
!  E119
assign (resid 119 and name N) (resid 119 and name CA) (resid 119 and name C) (resid 120
and name N)  1.00  -35.00  20.00  2
!  E120
assign (resid 120 and name N) (resid 120 and name CA) (resid 120 and name C) (resid 121
and name N)  1.00  -35.00  20.00  2
!  I121
assign (resid 121 and name N) (resid 121 and name CA) (resid 121 and name C) (resid 122
and name N)  1.00  -35.00  20.00  2
!  D122
assign (resid 122 and name N) (resid 122 and name CA) (resid 122 and name C) (resid 123
and name N)  1.00  -35.00  20.00  2
!  E123
assign (resid 123 and name N) (resid 123 and name CA) (resid 123 and name C) (resid 124
and name N)  1.00  -35.00  20.00  2
!  M124
assign (resid 124 and name N) (resid 124 and name CA) (resid 124 and name C) (resid 125
and name N)  1.00  -35.00  20.00  2
!  I125
assign (resid 125 and name N) (resid 125 and name CA) (resid 125 and name C) (resid 126
and name N)  1.00  -35.00  20.00  2
!  R126
assign (resid 126 and name N) (resid 126 and name CA) (resid 126 and name C) (resid 127
and name N)  1.00  -35.00  20.00  2
!  E127
assign (resid 127 and name N) (resid 127 and name CA) (resid 127 and name C) (resid 128
and name N)  1.00  -35.00  20.00  2
!  D133
assign (resid 133 and name N) (resid 133 and name CA) (resid 133 and name C) (resid 134
and name N)  1.00  135.00  40.00  2
!  M135
assign (resid 135 and name N) (resid 135 and name CA) (resid 135 and name C) (resid 136
and name N)  1.00  135.00  20.00  2
!  I136
assign (resid 136 and name N) (resid 136 and name CA) (resid 136 and name C) (resid 137
and name N)  1.00  135.00  20.00  2
!  Y138
assign (resid 138 and name N) (resid 138 and name CA) (resid 138 and name C) (resid 139
and name N)  1.00  -35.00  20.00  2
!  E139
assign (resid 139 and name N) (resid 139 and name CA) (resid 139 and name C) (resid 140
and name N)  1.00  -35.00  20.00  2
!  F141
assign (resid 141 and name N) (resid 141 and name CA) (resid 141 and name C) (resid 142
and name N)  1.00  -35.00  20.00  2
!  V142
assign (resid 142 and name N) (resid 142 and name CA) (resid 142 and name C) (resid 143
and name N)  1.00  -35.00  20.00  2
!  W143
assign (resid 143 and name N) (resid 143 and name CA) (resid 143 and name C) (resid 144
and name N)  1.00  -35.00  20.00  2
!  M144
```

```
assign (resid 144 and name N) (resid 144 and name CA) (resid 144 and name C) (resid 145
and name N)  1.00  -35.00  20.00  2
!  I145
assign (resid 145 and name N) (resid 145 and name CA) (resid 145 and name C) (resid 146
and name N)  1.00  -35.00  20.00  2
```

C.4 Stereoassignments for apoN androcam

File name: "stereoassign.cns"

```
!$Revision: 2.1 $
!$Date: 2001/06/18 12:28:21 $
!$RCSfile: stereoassign.cns,v $
!!! SWAP
!!! methylene protons
for $id in id   (
{===>}   (name cb and resid 3)
{===>}or (name cb and resid 35)
{===>}or (name cb and resid 64)
{===>}or (name cb and resid 78)
{===>}or (name cb and resid 82)
{===>}or (name cb and resid 95)
{===>}or (name cb and resid 115)
{===>}or (name cb and resid 131)
{===>}or (name cb and resid 133)
!{===>}or (name cb and resid 138)
{===>}or (name cb and resid 147)     )
loop Lmethy
    display aria revert  (bondedto (id $id) and name h*)  end
    display do (store1=0) (bondedto (id $id) and name h*)
end loop Lmethy
!!! SWAP
!!! isopropyle groups
for $id in id (name ca and (
{===>}resid 142
))
loop Vrev
    display do (store1=0) (byresid (id $id) and (name cg1 or name cd1))
    show (resn) (id $id)
    if ($result eq VAL) then
      evaluate ($name3 = cg1)
      evaluate ($name4 = cg2)
    elseif ($result eq LEU) then
      evaluate ($name3 = cd1)
      evaluate ($name4 = cd2)
    end if
    display aria revert (bondedto (byresid(id $id)
    display and (name $name3 or name $name4)) and name h*) end
end loop Vrev
!!! AS IS
!!! methylene protons
for $id in id   (
{===>}   (name cb and resid 7)
{===>}or (name cb and resid 14)
{===>}or (name cb and resid 20)
{===>}or (name cb and resid 49)
{===>}or (name cb and resid 93)
{===>}or (name cb and resid 105)
{===>}or (name cb and resid 124)
{===>}or (name cb and resid 126)
{===>}or (name cb and resid 129)
{===>}or (name cb and resid 141)
{===>}or (name cb and resid 143)     )
loop Lmethy
    display do (store1=0) (bondedto (id $id) and name h*)
end loop Lmethy   ! typo in original ARIA file, changed by MKJ 2008-09-09
!!! AS IS
!!! isopropyle groups
for $id in id (name ca and
(
{===>}   resid 108
))
loop Vrev
    display do (store1=0) (byresid (id $id) and (name cg1 or name cd1))
end loop Vrev
```

C.5 CSI input / output files for apoN androcam

CSI input
File name : "csi.apoN-AcaM"

```
>Androcam (Drosophila Melanogaster)
>M. Joshi, K. R. MacKenzie (2010)
```

#	AA	HA	CA	CB	CO
1	M	4.21	55.7	29.4	177.1
2	S	4.00	57.8	64.0	174.7
3	E	4.36	56.1	30.7	176.3
4	L	4.56	54.4	43.2	177.9
5	T	4.41	60.3	71.3	175.4
6	E	3.89	59.9	29.3	179.5
7	E	4.00	60.2	29.0	179.3
8	Q	3.74	58.7	29.2	177.3
9	I	3.36	65.8	37.6	177.8
10	A	4.10	55.4	17.9	180.4
11	E	4.15	59.3	29.5	180.4
12	F	4.89	57.6	36.6	178.5
13	K	3.69	59.9	31.4	177.6
14	D	4.40	57.3	40.8	178.4
15	A	4.35	54.8	19.5	179.0
16	F	3.78	62.4	41.0	177.9
17	V	3.88	65.4	31.7	178.6
18	Q	3.93	57.8	27.9	177.0
19	F	4.24	58.7	39.7	174.9
20	D	4.91	52.2	39.7	176.8
21	K	4.02	58.9	32.5	178.7
22	E	4.38	56.2	30.1	177.2
23	G	3.87	47.4	0	176.1
24	T	4.32	62.3	70.7	176.8
25	G	4.05	45.4	0	173.6
26	K	5.41	54.6	37.1	174.6
27	I	4.55	58.3	41.6	174.9
28	A	4.80	51.4	19.4	179.7
29	T	3.59	65.8	67.3	177.4
30	R	4.22	58.1	29.4	177.0
31	E	4.62	55.9	29.9	176.0
32	L	3.66	58.6	42.1	178.0
33	G	3.62	48.2	0	175.2
34	T	3.82	66.1	68.4	176.9
35	L	3.66	58.4	40.2	178.6
36	M	3.74	61.3	32.9	178.7
37	R	4.61	59.3	30.2	181.6
38	T	4.19	66.3	69.2	175.4
39	L	4.57	54.4	42.7	177.0
40	G	4.02	46.0	0	174.4
41	Q	4.42	53.6	30.2	173.9
42	N	5.17	51.1	39.5	171.9
43	P	4.71	62.4	31.6	177.6
44	T	4.36	61.0	70.8	175.1
45	E	3.97	60.0	29.1	179.1
46	A	4.06	55.0	18.3	180.5
47	E	4.01	58.8	29.9	180.0
48	L	3.92	58.0	41.9	178.3
49	Q	3.88	58.9	27.9	178.8
50	D	4.48	57.3	40.6	178.8
51	L	4.07	57.8	42.5	179.9
52	I	3.77	64.3	38.5	177.0
53	A	4.08	55.4	18.3	180.1
54	E	4.08	59.0	29.4	178.3
55	A	4.28	54.1	18.7	180.9
56	E	3.96	59.0	29.4	177.6
57	N	4.63	54.8	39.2	176.4
58	N	4.84	54.1	39.9	175.6
59	N	4.96	53.3	39.7	175.8
60	N	4.58	54.2	38.2	175.6

61	G	3.91	46.6	0	174.5
62	Q	5.34	54.2	33.6	174.5
63	L	4.98	53.7	46.2	175.9
64	N	5.65	50.9	39.3	175.8
65	F	3.61	60.0	38.9	177.4
66	T	3.60	67.0	68.4	177.9
67	E	3.96	58.9	29.8	180.2
68	F	4.08	61.6	39.2	176.4
69	C	3.34	65.1	26.5	176.5
70	G	3.76	47.1	0	176.5
71	I	3.57	65.2	38.3	178.2
72	M	3.97	56.1	30.4	178.7
73	A	3.98	55.1	18.0	180.0
74	K	4.02	58.7	32.5	178.3
75	Q	4.02	56.8	28.5	177.2
76	M	4.25	56.5	32.3	176.2
77	R	4.27	56.8	30.8	176.6
78	E	4.35	56.7	30.3	176.9
79	T	4.30	62.3	70.0	174.4
80	D	4.72	54.5	41.2	177.1
81	T	4.27	63.6	69.5	175.7
82	E	4.22	58.7	29.6	177.7
83	E	4.04	59.3	29.5	178.7
84	E	4.10	59.6	29.6	179.9
85	M	4.54	58.7	32.8	178.5
86	R	4.08	59.5	29.8	179.8
87	E	4.05	59.0	29.1	179.0
88	A	4.24	55.2	17.9	179.5
89	F	3.09	62.0	39.5	176.8
90	K	3.85	59.1	32.7	178.1
91	I	3.58	63.6	37.4	177.4
92	F	4.23	59.9	40.8	176.8
93	D	4.50	52.2	38.4	177.1
94	R	3.86	58.8	30.5	178.3
95	D	4.55	53.0	39.9	177.9
96	G	3.84	47.4	0	175.3
97	D	4.47	54.1	40.4	176.8
98	G	3.72	45.0	0	172.5
99	F	5.16	56.2	44.5	174.7
100	I	4.75	60.6	38.8	175.7
101	S	5.09	55.9	63.6	173.6
102	P	4.11	66.5	31.5	178.4
103	A	4.09	55.2	18.5	181.6
104	E	4.05	59.5	29.3	178.8
105	L	4.15	58.4	42.1	178.4
106	R	3.76	60.2	30.6	177.2
107	F	4.06	61.5	39.6	178.1
108	V	3.44	66.6	31.8	178.1
109	M	4.13	58.8	31.9	178.8
110	I	3.95	64.2	37.4	180.4
111	N	4.36	56.1	38.6	176.9
112	L	4.29	55.6	42.5	177.8
113	G	3.98	45.5	0	174.7
114	E	4.31	55.7	30.5	176.1
115	K	4.37	55.6	31.5	175.7
116	V	4.64	60.1	34.7	175.6
117	T	4.58	60.3	71.7	175.5
118	D	4.26	58.0	39.9	178.6
119	E	4.09	60.2	29.0	179.3
120	E	4.00	59.2	30.0	179.9
121	I	3.81	63.4	36.2	177.4
122	D	4.33	57.7	40.5	179.3
123	E	4.05	59.2	29.5	178.0
124	M	4.10	59.4	33.6	179.2
125	I	3.55	64.4	37.0	176.8
126	R	3.92	59.7	30.2	179.5
127	E	3.90	58.7	30.1	176.6
128	A	4.15	51.8	20.7	175.8
129	D	4.54	53.0	39.6	177.6
130	F	4.32	59.6	39.0	177.9
131	D	4.62	53.4	39.8	178.4

```
132  G    3.83      47.6      0         175.2
133  D    4.43      53.7      40.1      177.4
134  G    3.64      45.7      0         173.0
135  M    4.88      52.5      37.3      175.0
136  I    5.39      58.4      38.1      176.3
137  N    5.20      51.2      38.1      175.1
138  Y    3.55      63.0      37.8      175.9
139  E    3.66      60.6      29.1      180.5
140  E    4.12      58.8      29.8      178.4
141  F    4.06      61.4      39.5      176.3
142  V    3.21      66.5      31.6      179.5
143  W    4.04      62.0      28.6      178.6
144  M    3.71      59.6      34.1      179.0
145  I    3.85      61.8      37.3      177.6
146  S    4.29      59.7      63.7      174.3
147  Q    3.96      55.4      28.7      175.1
148  K    3.99      57.6      33.3      181.3
```

CSI output
File name : "apoN_ACaM.out"

```
################################################################################
#  Program...:                          CSI (c)
#  Version...:                           2.0
#  Location..:                  University of Alberta
#                          Protein Engineering Network of
#                               Centres of Excellence
#  Input.....:                  /data/mkjoshi/apoN_ACaM/CSI/library/csi/SAMPLE/csi.apoN-
ACaM
#  Date......:                  Mon Feb  1 11:17:12 2010
################################################################################
#     A       HA       CA       CO       CB       Consensus
#
1     M       0 C      0 C      0 C      0 C         0 C
2     S      -1 C      0 C      1 C      1 B         0 C
3     E       0 C      0 C      0 C      1 B         0 C
4     L       1 C     -1 C      1 C      1 B         0 C
5     T       0 C     -1 C      0 H      1 B         0 C
6     E      -1 H      1 H      1 H      0 C        -1 H
7     E      -1 H      1 H      1 H      0 C        -1 H
8     Q      -1 H      1 H      1 H     -1 C        -1 H
9     I      -1 H      1 H      1 H      0 C        -1 H
10    A      -1 H      1 H      1 H     -1 C        -1 H
11    E      -1 H      1 H      1 H      0 C        -1 H
12    F       1 C      0 H      1 H     -1 C        -1 H
13    K      -1 C      1 H      1 H     -1 C        -1 H
14    D      -1 C      1 H      1 H      0 C        -1 H
15    A       0 C      1 H      1 H      0 C        -1 H
16    F      -1 C      1 H      1 H      1 C        -1 H
17    V       0 C      1 H      1 H      0 C        -1 H
18    Q      -1 C      1 H      1 H     -1 C        -1 H
19    F      -1 C      1 H     -1 C      0 C         0 C
20    D       1 C     -1 C      0 C     -1 C         0 C
21    K      -1 C      1 C      1 H      0 C         0 C
22    E       0 C      0 C      1 H      0 C         0 C
23    G      -1 C      1 C      1 H     -1 C         0 C
24    T       0 C     -1 B      1 H      1 C         0 C
25    G       0 C      0 B      0 C      0 C         0 C
26    K       1 B     -1 B     -1 C      1 C         1 B
27    I       1 B     -1 B     -1 C      1 C         1 B
28    A       1 B     -1 B      1 H      0 C         1 B
29    T      -1 C      1 C      1 H     -1 C         0 C
30    R      -1 C      1 C      1 H     -1 C         0 C
31    E       1 C     -1 C      0 H      0 C         0 C
32    L      -1 H      1 H      1 H      0 C        -1 H
33    G      -1 H      1 H      1 H     -1 C        -1 H
34    T      -1 H      1 H      1 H      0 C        -1 H
35    L      -1 H      1 H      1 H     -1 C        -1 H
36    M      -1 H      1 H      1 H      0 C        -1 H
37    R       1 C      1 H      1 H      0 C        -1 H
```

38	T	-1	C	1	H	0	C	1	C	0	C
39	L	1	C	-1	C	0	C	1	C	0	C
40	G	0	C	1	C	1	C	0	C	0	C
41	Q	0	C	-1	C	-1	C	0	C	0	C
42	N	1	C	-1	C	-1	C	0	C	0	C
43	P	1	C	0	C	0	C	0	C	0	C
44	T	0	C	-1	C	0	C	1	C	0	C
45	E	-1	H	1	H	1	H	0	C	-1	H
46	A	-1	H	1	H	1	H	0	C	-1	H
47	E	-1	H	1	H	1	H	0	C	-1	H
48	L	-1	H	1	H	1	H	0	C	-1	H
49	Q	-1	H	1	H	1	H	-1	C	-1	H
50	D	-1	H	1	H	1	H	0	C	-1	H
51	L	0	H	1	H	1	H	0	C	-1	H
52	I	-1	H	1	H	0	H	1	C	-1	H
53	A	-1	H	1	H	1	H	0	C	-1	H
54	E	-1	H	1	H	1	H	0	C	-1	H
55	A	0	H	1	H	1	H	0	C	-1	H
56	E	-1	H	1	H	1	H	0	C	-1	H
57	N	-1	H	1	H	1	H	0	C	-1	H
58	N	0	C	0	C	0	H	1	C	0	C
59	N	1	C	0	C	1	C	1	C	0	C
60	N	-1	C	0	C	0	C	-1	C	0	C
61	G	0	C	1	C	1	C	0	C	0	C
62	Q	1	B	-1	B	-1	C	1	C	1	B
63	L	1	B	-1	B	-1	C	1	C	1	B
64	N	1	B	-1	B	1	H	0	C	1	B
65	F	-1	H	1	H	1	H	0	C	-1	H
66	T	-1	H	1	H	1	H	0	C	-1	H
67	E	-1	H	1	H	1	H	0	C	-1	H
68	F	-1	H	1	H	1	H	0	C	-1	H
69	C	-1	H	1	H	1	H	-1	C	-1	H
70	G	-1	H	1	H	1	H	-1	C	-1	H
71	I	-1	H	1	H	1	H	1	C	-1	H
72	M	-1	H	0	H	1	H	-1	C	-1	H
73	A	-1	H	1	H	1	H	-1	C	-1	H
74	K	-1	H	1	H	1	H	0	C	-1	H
75	Q	-1	H	0	C	1	H	-1	C	-1	H
76	M	-1	H	0	C	0	C	0	C	0	C
77	R	-1	H	0	C	0	C	0	C	0	C
78	E	0	C	0	C	1	C	0	C	0	C
79	T	0	C	-1	C	-1	C	1	C	0	C
80	D	0	C	0	C	0	C	0	C	0	C
81	T	0	C	0	C	0	C	1	C	0	C
82	E	0	C	1	H	1	H	0	C	-1	H
83	E	-1	H	1	H	1	H	0	C	-1	H
84	E	-1	H	1	H	1	H	0	C	-1	H
85	M	0	H	1	H	1	H	0	C	-1	H
86	R	-1	H	1	H	1	H	0	C	-1	H
87	E	-1	H	1	H	1	H	0	C	-1	H
88	A	-1	H	1	H	1	H	-1	C	-1	H
89	F	-1	H	1	H	1	H	0	C	-1	H
90	K	-1	H	1	H	1	H	0	C	-1	H
91	I	-1	H	1	H	1	H	0	C	-1	H
92	F	-1	H	1	H	1	H	1	C	-1	H
93	D	-1	H	-1	C	0	H	-1	C	-1	H
94	R	-1	H	1	C	1	H	0	C	-1	H
95	D	-1	H	-1	C	1	H	-1	C	-1	H
96	G	-1	H	1	C	1	H	-1	C	-1	H
97	D	-1	H	0	C	0	C	0	C	0	C
98	G	-1	H	0	C	-1	B	-1	C	0	C
99	F	1	B	-1	B	-1	B	1	B	1	B
100	I	1	B	-1	B	-1	B	1	B	1	B
101	S	1	B	-1	B	0	C	1	B	1	B
102	P	-1	H	0	C	0	C	0	C	0	C
103	A	-1	H	1	H	1	H	0	C	-1	H
104	E	-1	H	1	H	1	H	0	C	-1	H
105	L	0	H	1	H	1	H	0	C	-1	H
106	R	-1	H	1	H	1	H	0	C	-1	H
107	F	-1	H	1	H	1	H	0	C	-1	H
108	V	-1	H	1	H	1	H	0	C	-1	H

```
109   M      -1 H      1 H      1 H     -1 C       -1 H
110   I       0 H      1 H      1 H      0 C       -1 H
111   N      -1 H      1 H      1 H      0 C       -1 H
112   L       1 C      0 C      1 H      0 C        0 C
113   G       0 C      0 C      1 H      0 C        0 C
114   E       0 C     -1 B      0 C      1 C        0 C
115   K       0 C     -1 B     -1 C     -1 C        0 C
116   V       1 C     -1 B     -1 C      1 C        0 C
117   T       1 C     -1 B      0 C      1 C        0 C
118   D      -1 H      1 H      1 H     -1 C       -1 H
119   E      -1 H      1 H      1 H      0 C       -1 H
120   E      -1 H      1 H      1 H      0 C       -1 H
121   I      -1 H      1 H      1 H     -1 C       -1 H
122   D      -1 H      1 H      1 H      0 C       -1 H
123   E      -1 H      1 H      1 H      0 C       -1 H
124   M      -1 H      1 H      1 H      1 C       -1 H
125   I      -1 H      1 H      0 H      0 C       -1 H
126   R      -1 H      1 H      1 H      0 C       -1 H
127   E      -1 H      1 H      1 H      0 C       -1 H
128   A      -1 H     -1 C     -1 C      1 C        0 C
129   D      -1 H     -1 C      0 C     -1 C        0 C
130   F      -1 H      1 C      1 C      0 C        0 C
131   D      -1 H     -1 C      1 C     -1 C        0 C
132   G      -1 H      1 C      1 C     -1 C        0 C
133   D      -1 H      0 C      0 C      0 C        0 C
134   G      -1 H      1 C     -1 B     -1 C        0 C
135   M       1 B     -1 B     -1 B      1 C        1 B
136   I       1 B     -1 B     -1 B      0 C        1 B
137   N       1 B     -1 B      0 C     -1 C        1 B
138   Y      -1 H      1 H      0 C     -1 C       -1 H
139   E      -1 H      1 H      1 H      0 C       -1 H
140   E      -1 H      1 H      1 H      0 C       -1 H
141   F      -1 H      1 H      1 H      0 C       -1 H
142   V      -1 H      1 H      1 H      0 C       -1 H
143   W      -1 H      1 H      1 H      0 C       -1 H
144   M      -1 H      1 H      1 H      1 C       -1 H
145   I      -1 H     -1 C      1 H      0 C       -1 H
146   S      -1 H      1 C      1 H      1 C       -1 H
147   Q      -1 H     -1 C     -1 C     -1 C        0 C
148   K       0 C      0 C      0 C      0 C        0 C
#
##############################################################################
#                    Secondary Structure Summary
#
#       HA      |       CA       |       CO       |       CB       | Consensus |
#---------------------------------------------------------------------------------
# C   1 -   5   | C   1 -   5    | C   1 -   4    | C   1 -   1    | C   0 -   0 |
# H   6 -  11   | H   6 -  19    | H   5 -  18    | B   2 -   5    | H   0 -   0 |
# C  12 -  25   | C  20 -  23    | C  19 -  20    | C   6 -  98    | C   0 -   0 |
# B  26 -  28   | B  24 -  28    | H  21 -  24    | B  99 - 101    | B   0 -   0 |
# C  29 -  31   | C  29 -  31    | C  25 -  27    | C 102 - 148    | C   0 -   0 |
# H  32 -  36   | H  32 -  38    | H  28 -  37    |                | H   0 -   0 |
# C  37 -  44   | C  39 -  44    | C  38 -  44    |                | C   0 -   0 |
# H  45 -  57   | H  45 -  57    | H  45 -  58    |                | H   0 -   0 |
# C  58 -  61   | C  58 -  61    | C  59 -  63    |                | C   0 -   0 |
# B  62 -  64   | B  62 -  64    | H  64 -  75    |                | B   0 -   0 |
# H  65 -  77   | H  65 -  74    | C  76 -  81    |                | H   0 -   0 |
# C  78 -  82   | C  75 -  81    | H  82 -  96    |                | C   0 -   0 |
# H  83 -  98   | H  82 -  92    | C  97 -  97    |                | H   0 -   0 |
# B  99 - 101   | C  93 -  98    | B  98 - 100    |                | C   0 -   0 |
# H 102 - 111   | B  99 - 101    | C 101 - 102    |                | B   0 -   0 |
# C 112 - 117   | C 102 - 102    | H 103 - 113    |                | C   0 -   0 |
# H 118 - 134   | H 103 - 111    | C 114 - 117    |                | H   0 -   0 |
# B 135 - 137   | C 112 - 113    | H 118 - 127    |                | C   0 -   0 |
# H 138 - 147   | B 114 - 117    | C 128 - 133    |                | H   0 -   0 |
# C 148 - 148   | H 118 - 127    | B 134 - 136    |                | C   0 -   0 |
#               | C 128 - 134    | C 137 - 138    |                | B   0 -   0 |
#               | B 135 - 137    | H 139 - 146    |                | H   0 -   0 |
#               | H 138 - 144    | C 147 - 148    |                | C   0 -   0 |
#               | C 145 - 148    |                |                |             |
```

C.6 Karplus restraints derived from $^3J_{HNHA}$ for apoN androcam

File name : "phi_karp.tbl"

```
! Karplus restraints for phi from 3D HNHA data derived on 2010-02-03
!   S2
assign (resid   1 and name C ) (resid   2 and name N )
       (resid   2 and name CA) (resid   2 and name HA )    4.55     0.50
!   L4
assign (resid   3 and name C ) (resid   4 and name N )
       (resid   4 and name CA) (resid   4 and name HA )    6.31     0.50
!   T5
assign (resid   4 and name C ) (resid   5 and name N )
       (resid   5 and name CA) (resid   5 and name HA )    8.05     0.50
!   E6
assign (resid   5 and name C ) (resid   6 and name N )
       (resid   6 and name CA) (resid   6 and name HA )    2.64     0.50
!   E7
assign (resid   6 and name C ) (resid   7 and name N )
       (resid   7 and name CA) (resid   7 and name HA )    4.61     0.50
!   Q8
assign (resid   7 and name C ) (resid   8 and name N )
       (resid   8 and name CA) (resid   8 and name HA )    3.87     0.50
!   I9
assign (resid   8 and name C ) (resid   9 and name N )
       (resid   9 and name CA) (resid   9 and name HA )    4.78     0.50
!   A10
assign (resid   9 and name C ) (resid   10 and name N )
       (resid   10 and name CA) (resid   10 and name HA )    3.98     0.50
!   E11
assign (resid   10 and name C ) (resid   11 and name N )
       (resid   11 and name CA) (resid   11 and name HA )    5.38     0.50
!   F12
assign (resid   11 and name C ) (resid   12 and name N )
       (resid   12 and name CA) (resid   12 and name HA )    4.07     0.50
!   K13
assign (resid   12 and name C ) (resid   13 and name N )
       (resid   13 and name CA) (resid   13 and name HA )    4.40     0.50
!   A15
assign (resid   14 and name C ) (resid   15 and name N )
       (resid   15 and name CA) (resid   15 and name HA )    5.07     0.50
!   F16
assign (resid   15 and name C ) (resid   16 and name N )
       (resid   16 and name CA) (resid   16 and name HA )    2.60     0.50
!   V17
assign (resid   16 and name C ) (resid   17 and name N )
       (resid   17 and name CA) (resid   17 and name HA )    3.14     0.50
!   Q18
assign (resid   17 and name C ) (resid   18 and name N )
       (resid   18 and name CA) (resid   18 and name HA )    5.28     0.50
!   F19
assign (resid   18 and name C ) (resid   19 and name N )
       (resid   19 and name CA) (resid   19 and name HA )    8.67     0.50
!   D20
assign (resid   19 and name C ) (resid   20 and name N )
       (resid   20 and name CA) (resid   20 and name HA )    8.12     0.50
!   K21
assign (resid   20 and name C ) (resid   21 and name N )
       (resid   21 and name CA) (resid   21 and name HA )    4.51     0.50
!   E22
assign (resid   21 and name C ) (resid   22 and name N )
       (resid   22 and name CA) (resid   22 and name HA )    7.51     0.50
!   T24
assign (resid   23 and name C ) (resid   24 and name N )
       (resid   24 and name CA) (resid   24 and name HA )    8.59     0.50
!   I27
assign (resid   26 and name C ) (resid   27 and name N )
       (resid   27 and name CA) (resid   27 and name HA )    9.92     0.50
!   A28
```

```
assign (resid   27 and name C ) (resid   28 and name N )
       (resid   28 and name CA) (resid   28 and name HA )    6.02     0.50
!   T29
assign (resid   28 and name C ) (resid   29 and name N )
       (resid   29 and name CA) (resid   29 and name HA )    3.42     0.50
!   R30
assign (resid   29 and name C ) (resid   30 and name N )
       (resid   30 and name CA) (resid   30 and name HA )    4.85     0.50
!   L32
assign (resid   31 and name C ) (resid   32 and name N )
       (resid   32 and name CA) (resid   32 and name HA )    6.83     0.50
!   T34
assign (resid   33 and name C ) (resid   34 and name N )
       (resid   34 and name CA) (resid   34 and name HA )    3.65     0.50
!   L35
assign (resid   34 and name C ) (resid   35 and name N )
       (resid   35 and name CA) (resid   35 and name HA )    4.67     0.50
!   M36
assign (resid   35 and name C ) (resid   36 and name N )
       (resid   36 and name CA) (resid   36 and name HA )    3.65     0.50
!   R37
assign (resid   36 and name C ) (resid   37 and name N )
       (resid   37 and name CA) (resid   37 and name HA )    4.62     0.50
!   T38
assign (resid   37 and name C ) (resid   38 and name N )
       (resid   38 and name CA) (resid   38 and name HA )    3.39     0.50
!   L39
assign (resid   38 and name C ) (resid   39 and name N )
       (resid   39 and name CA) (resid   39 and name HA )    8.00     0.50
!   Q41
assign (resid   40 and name C ) (resid   41 and name N )
       (resid   41 and name CA) (resid   41 and name HA )    8.75     0.50
!   N42
assign (resid   41 and name C ) (resid   42 and name N )
       (resid   42 and name CA) (resid   42 and name HA )    9.05     0.50
!   T44
assign (resid   43 and name C ) (resid   44 and name N )
       (resid   44 and name CA) (resid   44 and name HA )    7.30     0.50
!   E45
assign (resid   44 and name C ) (resid   45 and name N )
       (resid   45 and name CA) (resid   45 and name HA )    2.61     0.50
!   A46
assign (resid   45 and name C ) (resid   46 and name N )
       (resid   46 and name CA) (resid   46 and name HA )    4.21     0.50
!   L48
assign (resid   47 and name C ) (resid   48 and name N )
       (resid   48 and name CA) (resid   48 and name HA )    3.99     0.50
!   Q49
assign (resid   48 and name C ) (resid   49 and name N )
       (resid   49 and name CA) (resid   49 and name HA )    3.33     0.50
!   D50
assign (resid   49 and name C ) (resid   50 and name N )
       (resid   50 and name CA) (resid   50 and name HA )    4.96     0.50
!   L51
assign (resid   50 and name C ) (resid   51 and name N )
       (resid   51 and name CA) (resid   51 and name HA )    4.79     0.50
!   I52
assign (resid   51 and name C ) (resid   52 and name N )
       (resid   52 and name CA) (resid   52 and name HA )    6.32     0.50
!   A53
assign (resid   52 and name C ) (resid   53 and name N )
       (resid   53 and name CA) (resid   53 and name HA )    3.31     0.50
!   E54
assign (resid   53 and name C ) (resid   54 and name N )
       (resid   54 and name CA) (resid   54 and name HA )    3.85     0.50
!   A55
assign (resid   54 and name C ) (resid   55 and name N )
       (resid   55 and name CA) (resid   55 and name HA )    4.47     0.50
!   E56
assign (resid   55 and name C ) (resid   56 and name N )
       (resid   56 and name CA) (resid   56 and name HA )    5.24     0.50
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!   N57
assign (resid   56 and name C ) (resid   57 and name N )
       (resid   57 and name CA) (resid   57 and name HA )    7.54     0.50
!   N60
assign (resid   59 and name C ) (resid   60 and name N )
       (resid   60 and name CA) (resid   60 and name HA )    5.27     0.50
!   Q62
assign (resid   61 and name C ) (resid   62 and name N )
       (resid   62 and name CA) (resid   62 and name HA )    9.54     0.50
!   L63
assign (resid   62 and name C ) (resid   63 and name N )
       (resid   63 and name CA) (resid   63 and name HA )    9.33     0.50
!   N64
assign (resid   63 and name C ) (resid   64 and name N )
       (resid   64 and name CA) (resid   64 and name HA )    9.86     0.50
!   F65
assign (resid   64 and name C ) (resid   65 and name N )
       (resid   65 and name CA) (resid   65 and name HA )    3.25     0.50
!   T66
assign (resid   65 and name C ) (resid   66 and name N )
       (resid   66 and name CA) (resid   66 and name HA )    5.00     0.50
!   E67
assign (resid   66 and name C ) (resid   67 and name N )
       (resid   67 and name CA) (resid   67 and name HA )    4.75     0.50
!   F68
assign (resid   67 and name C ) (resid   68 and name N )
       (resid   68 and name CA) (resid   68 and name HA )    3.54     0.50
!   C69
assign (resid   68 and name C ) (resid   69 and name N )
       (resid   69 and name CA) (resid   69 and name HA )    2.42     0.50
!   I71
assign (resid   70 and name C ) (resid   71 and name N )
       (resid   71 and name CA) (resid   71 and name HA )    5.47     0.50
!   M72
assign (resid   71 and name C ) (resid   72 and name N )
       (resid   72 and name CA) (resid   72 and name HA )    4.11     0.50
!   A73
assign (resid   72 and name C ) (resid   73 and name N )
       (resid   73 and name CA) (resid   73 and name HA )    3.24     0.50
!   K74
assign (resid   73 and name C ) (resid   74 and name N )
       (resid   74 and name CA) (resid   74 and name HA )    4.71     0.50
!   Q75
assign (resid   74 and name C ) (resid   75 and name N )
       (resid   75 and name CA) (resid   75 and name HA )    5.49     0.50
!   M76
assign (resid   75 and name C ) (resid   76 and name N )
       (resid   76 and name CA) (resid   76 and name HA )    6.24     0.50
!   R77
assign (resid   76 and name C ) (resid   77 and name N )
       (resid   77 and name CA) (resid   77 and name HA )    4.30     0.50
!   E78
assign (resid   77 and name C ) (resid   78 and name N )
       (resid   78 and name CA) (resid   78 and name HA )    6.46     0.50
!   T79
assign (resid   78 and name C ) (resid   79 and name N )
       (resid   79 and name CA) (resid   79 and name HA )    6.76     0.50
!   D80
assign (resid   79 and name C ) (resid   80 and name N )
       (resid   80 and name CA) (resid   80 and name HA )    6.41     0.50
!   T81
assign (resid   80 and name C ) (resid   81 and name N )
       (resid   81 and name CA) (resid   81 and name HA )    5.96     0.50
!   E82
assign (resid   81 and name C ) (resid   82 and name N )
       (resid   82 and name CA) (resid   82 and name HA )    5.76     0.50
!   E83
assign (resid   82 and name C ) (resid   83 and name N )
       (resid   83 and name CA) (resid   83 and name HA )    3.91     0.50
!   E84
assign (resid   83 and name C ) (resid   84 and name N )
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       (resid   84 and name CA) (resid   84 and name HA )    3.71     0.50
!   M85
assign (resid   84 and name C ) (resid   85 and name N )
       (resid   85 and name CA) (resid   85 and name HA )    5.50     0.50
!   R86
assign (resid   85 and name C ) (resid   86 and name N )
       (resid   86 and name CA) (resid   86 and name HA )    4.25     0.50
!   E87
assign (resid   86 and name C ) (resid   87 and name N )
       (resid   87 and name CA) (resid   87 and name HA )    5.54     0.50
!   A88
assign (resid   87 and name C ) (resid   88 and name N )
       (resid   88 and name CA) (resid   88 and name HA )    7.27     0.50
!   F89
assign (resid   88 and name C ) (resid   89 and name N )
       (resid   89 and name CA) (resid   89 and name HA )    3.16     0.50
!   K90
assign (resid   89 and name C ) (resid   90 and name N )
       (resid   90 and name CA) (resid   90 and name HA )    4.48     0.50
!   I91
assign (resid   90 and name C ) (resid   91 and name N )
       (resid   91 and name CA) (resid   91 and name HA )    5.09     0.50
!   F92
assign (resid   91 and name C ) (resid   92 and name N )
       (resid   92 and name CA) (resid   92 and name HA )    6.31     0.50
!   D93
assign (resid   92 and name C ) (resid   93 and name N )
       (resid   93 and name CA) (resid   93 and name HA )    5.74     0.50
!   R94
assign (resid   93 and name C ) (resid   94 and name N )
       (resid   94 and name CA) (resid   94 and name HA )    3.37     0.50
!   D95
assign (resid   94 and name C ) (resid   95 and name N )
       (resid   95 and name CA) (resid   95 and name HA )    8.43     0.50
!   D97
assign (resid   96 and name C ) (resid   97 and name N )
       (resid   97 and name CA) (resid   97 and name HA )    7.60     0.50
!   F99
assign (resid   98 and name C ) (resid   99 and name N )
       (resid   99 and name CA) (resid   99 and name HA )    9.43     0.50
!   I100
assign (resid    99 and name C ) (resid   100 and name N )
       (resid   100 and name CA) (resid   100 and name HA )    8.32     0.50
!   S101
assign (resid   100 and name C ) (resid   101 and name N )
       (resid   101 and name CA) (resid   101 and name HA )    6.51     0.50
!   A103
assign (resid   102 and name C ) (resid   103 and name N )
       (resid   103 and name CA) (resid   103 and name HA )    4.12     0.50
!   E104
assign (resid   103 and name C ) (resid   104 and name N )
       (resid   104 and name CA) (resid   104 and name HA )    6.00     0.50
!   L105
assign (resid   104 and name C ) (resid   105 and name N )
       (resid   105 and name CA) (resid   105 and name HA )    5.09     0.50
!   R106
assign (resid   105 and name C ) (resid   106 and name N )
       (resid   106 and name CA) (resid   106 and name HA )    3.89     0.50
!   F107
assign (resid   106 and name C ) (resid   107 and name N )
       (resid   107 and name CA) (resid   107 and name HA )    2.79     0.50
!   V108
assign (resid   107 and name C ) (resid   108 and name N )
       (resid   108 and name CA) (resid   108 and name HA )    8.43     0.50
!   M109
assign (resid   108 and name C ) (resid   109 and name N )
       (resid   109 and name CA) (resid   109 and name HA )    4.93     0.50
!   I110
assign (resid   109 and name C ) (resid   110 and name N )
       (resid   110 and name CA) (resid   110 and name HA )    7.55     0.50
!   N111
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assign (resid   110 and name C ) (resid   111 and name N )
       (resid   111 and name CA) (resid   111 and name HA )    5.34      0.50
!   L112
assign (resid   111 and name C ) (resid   112 and name N )
       (resid   112 and name CA) (resid   112 and name HA )    4.96      0.50
!   E114
assign (resid   113 and name C ) (resid   114 and name N )
       (resid   114 and name CA) (resid   114 and name HA )    7.82      0.50
!   K115
assign (resid   114 and name C ) (resid   115 and name N )
       (resid   115 and name CA) (resid   115 and name HA )    7.59      0.50
!   V116
assign (resid   115 and name C ) (resid   116 and name N )
       (resid   116 and name CA) (resid   116 and name HA )    8.67      0.50
!   T117
assign (resid   116 and name C ) (resid   117 and name N )
       (resid   117 and name CA) (resid   117 and name HA )    7.98      0.50
!   D118
assign (resid   117 and name C ) (resid   118 and name N )
       (resid   118 and name CA) (resid   118 and name HA )    3.23      0.50
!   E119
assign (resid   118 and name C ) (resid   119 and name N )
       (resid   119 and name CA) (resid   119 and name HA )    3.52      0.50
!   E120
assign (resid   119 and name C ) (resid   120 and name N )
       (resid   120 and name CA) (resid   120 and name HA )    5.63      0.50
!   I121
assign (resid   120 and name C ) (resid   121 and name N )
       (resid   121 and name CA) (resid   121 and name HA )    4.32      0.50
!   D122
assign (resid   121 and name C ) (resid   122 and name N )
       (resid   122 and name CA) (resid   122 and name HA )    3.31      0.50
!   E123
assign (resid   122 and name C ) (resid   123 and name N )
       (resid   123 and name CA) (resid   123 and name HA )    4.54      0.50
!   M124
assign (resid   123 and name C ) (resid   124 and name N )
       (resid   124 and name CA) (resid   124 and name HA )    3.77      0.50
!   I125
assign (resid   124 and name C ) (resid   125 and name N )
       (resid   125 and name CA) (resid   125 and name HA )    3.20      0.50
!   R126
assign (resid   125 and name C ) (resid   126 and name N )
       (resid   126 and name CA) (resid   126 and name HA )    2.33      0.50
!   E127
assign (resid   126 and name C ) (resid   127 and name N )
       (resid   127 and name CA) (resid   127 and name HA )    4.14      0.50
!   A128
assign (resid   127 and name C ) (resid   128 and name N )
       (resid   128 and name CA) (resid   128 and name HA )    8.90      0.50
!   D129
assign (resid   128 and name C ) (resid   129 and name N )
       (resid   129 and name CA) (resid   129 and name HA )    5.16      0.50
!   F130
assign (resid   129 and name C ) (resid   130 and name N )
       (resid   130 and name CA) (resid   130 and name HA )    5.21      0.50
!   D133
assign (resid   132 and name C ) (resid   133 and name N )
       (resid   133 and name CA) (resid   133 and name HA )   10.23      0.50
!   M135
assign (resid   134 and name C ) (resid   135 and name N )
       (resid   135 and name CA) (resid   135 and name HA )    8.63      0.50
!   I136
assign (resid   135 and name C ) (resid   136 and name N )
       (resid   136 and name CA) (resid   136 and name HA )    9.68      0.50
!   N137
assign (resid   136 and name C ) (resid   137 and name N )
       (resid   137 and name CA) (resid   137 and name HA )    8.32      0.50
!   Y138
assign (resid   137 and name C ) (resid   138 and name N )
       (resid   138 and name CA) (resid   138 and name HA )    2.09      0.50
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!   E139
assign (resid   138 and name C ) (resid   139 and name N )
       (resid   139 and name CA) (resid   139 and name HA )    4.64     0.50
!   E140
assign (resid   139 and name C ) (resid   140 and name N )
       (resid   140 and name CA) (resid   140 and name HA )    6.32     0.50
!   F141
assign (resid   140 and name C ) (resid   141 and name N )
       (resid   141 and name CA) (resid   141 and name HA )    4.41     0.50
!   V142
assign (resid   141 and name C ) (resid   142 and name N )
       (resid   142 and name CA) (resid   142 and name HA )    2.99     0.50
!   W143
assign (resid   142 and name C ) (resid   143 and name N )
       (resid   143 and name CA) (resid   143 and name HA )    5.59     0.50
!   M144
assign (resid   143 and name C ) (resid   144 and name N )
       (resid   144 and name CA) (resid   144 and name HA )    1.98     0.50
!   S146
assign (resid   145 and name C ) (resid   146 and name N )
       (resid   146 and name CA) (resid   146 and name HA )    6.98     0.50
!   Q147
assign (resid   146 and name C ) (resid   147 and name N )
       (resid   147 and name CA) (resid   147 and name HA )    8.42     0.50
!   K148
assign (resid   147 and name C ) (resid   148 and name N )
       (resid   148 and name CA) (resid   148 and name HA )    7.44     0.50

! Karplus restraints for phi from 3D HNHA data for Glycines derived on 2010-02-03
!   G23
!assign (resid   22 and name C ) (resid   23 and name N )
!       (resid   23 and name CA) (resid   23 and name HA# )    7.15     0.50
!   G25
assign (resid   24 and name C ) (resid   25 and name N )
       (resid   25 and name CA) (resid   25 and name HA1 )    4.89     0.50
!   G25
assign (resid   24 and name C ) (resid   25 and name N )
       (resid   25 and name CA) (resid   25 and name HA2 )    6.47     0.50
!   G33
assign (resid   32 and name C ) (resid   33 and name N )
       (resid   33 and name CA) (resid   33 and name HA2 )    5.20     0.50
!   G33
assign (resid   32 and name C ) (resid   33 and name N )
       (resid   33 and name CA) (resid   33 and name HA1 )    3.25     0.50
!   G40
assign (resid   39 and name C ) (resid   40 and name N )
       (resid   40 and name CA) (resid   40 and name HA2 )    6.00     0.50
!   G40
assign (resid   39 and name C ) (resid   40 and name N )
       (resid   40 and name CA) (resid   40 and name HA1 )    3.96     0.50
!   G70
!assign (resid   69 and name C ) (resid   70 and name N )
!       (resid   70 and name CA) (resid   70 and name HA# )    6.73     0.50
!   G96
!assign (resid   95 and name C ) (resid   96 and name N )
!       (resid   96 and name CA) (resid   96 and name HA# )    6.96     0.50
!   G98
assign (resid   97 and name C ) (resid   98 and name N )
       (resid   98 and name CA) (resid   98 and name HA1 )    5.63     0.50
!   G98
assign (resid   97 and name C ) (resid   98 and name N )
       (resid   98 and name CA) (resid   98 and name HA2 )    9.59     0.50
!   G113
assign (resid   112 and name C ) (resid   113 and name N )
       (resid   113 and name CA) (resid   113 and name HA1 )    5.76     0.50
!   G113
assign (resid   112 and name C ) (resid   113 and name N )
       (resid   113 and name CA) (resid   113 and name HA2 )    7.12     0.50
!   G132
assign (resid   131 and name C ) (resid   132 and name N )
       (resid   132 and name CA) (resid   132 and name HA1 )    5.83     0.50
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!   G132
assign (resid   131 and name C ) (resid   132 and name N )
       (resid   132 and name CA) (resid   132 and name HA2 )    2.90     0.50
!   G134
assign (resid   133 and name C ) (resid   134 and name N )
       (resid   134 and name CA) (resid   134 and name HA1 )    5.00     0.50
!   G134
assign (resid   133 and name C ) (resid   134 and name N )
       (resid   134 and name CA) (resid   134 and name HA2 )    6.30     0.50
```

C.7 J couplings for apoN androcam

C.7.1 $^3J_{HNHA}$

Residue	HN auto peak volume	Hα cross peak volume	$^3J_{HNHA}$				
S2	6.39E+08	8.90E+07	4.55	T44	1.03E+09	4.30E+08	7.30
L4	1.83E+09	5.35E+08	6.31	E45	1.25E+09	5.39E+07	2.61
T5	8.28E+08	4.44E+08	8.05	A46	1.73E+09	2.04E+08	4.21
E6	1.48E+09	6.53E+07	2.64	L48	1.42E+09	1.49E+08	3.99
E7	1.76E+09	2.53E+08	4.61	Q49	1.97E+09	1.41E+08	3.33
Q8	2.11E+09	2.08E+08	3.87	D50	1.65E+09	2.78E+08	4.96
I9	1.15E+09	1.79E+08	4.78	L51	1.44E+09	2.25E+08	4.79
A10	2.14E+09	2.24E+08	3.98	I52	5.12E+08	1.50E+08	6.32
E11	1.89E+09	3.82E+08	5.38	A53	1.71E+09	1.21E+08	3.31
F12	1.65E+09	1.81E+08	4.07	E54	1.38E+09	1.34E+08	3.85
K13	7.58E+08	9.83E+07	4.40	A55	7.61E+08	1.02E+08	4.47
A15	3.62E+09	6.40E+08	5.07	E56	9.19E+08	1.75E+08	5.24
F16	1.31E+09	5.64E+07	2.60	N57	5.87E+08	2.66E+08	7.54
V17	1.34E+09	8.51E+07	3.14	N60	3.57E+08	6.88E+07	5.27
Q18	1.65E+09	3.20E+08	5.28	Q62	2.01E+08	1.74E+08	9.54
F19	8.52E+08	5.59E+08	8.67	L63	6.22E+08	5.04E+08	9.33
D20	1.30E+09	7.14E+08	8.12	N64	4.55E+08	4.35E+08	9.86
K21	2.92E+09	4.00E+08	4.51	F65	7.57E+08	5.17E+07	3.25
E22	1.38E+09	6.19E+08	7.51	T66	1.25E+09	2.15E+08	5.00
T24	1.05E+09	6.72E+08	8.59	E67	9.86E+08	1.51E+08	4.75
G25 HA1	8.33E+08	4.00E+07	2.75	F68	9.05E+08	7.38E+07	3.54
G25 HA2	8.33E+08	8.32E+07	3.90	C69	1.13E+09	4.17E+07	2.42
I27	5.40E+08	5.26E+08	9.92	I71	9.28E+08	1.95E+08	5.47
A28	1.35E+09	3.53E+08	6.02	M72	8.68E+08	9.69E+07	4.11
T29	4.83E+08	3.67E+07	3.42	A73	1.23E+09	8.35E+07	3.24
R30	6.93E+08	1.11E+08	4.85	K74	2.11E+09	3.17E+08	4.71
L32	1.43E+08	5.06E+07	6.83	Q75	1.02E+09	2.16E+08	5.49
G33 HA1	1.10E+09	7.50E+07	3.25	M76	8.72E+08	2.48E+08	6.24
G33 HA2	1.10E+09	2.06E+08	5.20	R77	2.41E+09	2.98E+08	4.30
T34	1.45E+09	1.26E+08	3.65	E78	1.01E+09	3.12E+08	6.46
L35	1.23E+09	1.82E+08	4.67	T79	1.37E+09	4.72E+08	6.76
M36	7.81E+08	6.80E+07	3.65	D80	1.38E+09	4.18E+08	6.41
R37	1.35E+09	1.95E+08	4.62	T81	1.00E+09	2.56E+08	5.96
T38	1.55E+09	1.15E+08	3.39	E82	1.31E+09	3.10E+08	5.76
L39	1.30E+09	6.87E+08	8.00	E83	1.73E+09	1.74E+08	3.91
G40 HA1	1.20E+09	1.24E+08	3.96	E84	9.60E+08	8.65E+07	3.71
G40 HA2	1.20E+09	3.12E+08	6.00	M85	1.08E+09	2.30E+08	5.50
Q41	9.42E+08	6.34E+08	8.75	R86	9.90E+08	1.19E+08	4.25
N42	4.14E+08	3.07E+08	9.05	E87	1.11E+09	2.40E+08	5.54

A88	3.64E+08	1.50E+08	7.27	E119	2.10E+09	1.69E+08	3.52
F89	9.16E+08	5.88E+07	3.16	E120	1.24E+09	2.78E+08	5.63
K90	1.20E+09	1.62E+08	4.48	I121	1.30E+09	1.62E+08	4.32
I91	8.83E+08	1.58E+08	5.09	D122	2.42E+09	1.71E+08	3.31
F92	5.96E+08	1.74E+08	6.31	E123	1.95E+09	2.71E+08	4.54
D93	7.25E+08	1.70E+08	5.74	M124	1.58E+09	1.47E+08	3.77
R94	8.63E+08	6.35E+07	3.37	I125	7.67E+08	5.05E+07	3.20
D95	1.29E+09	7.85E+08	8.43	R126	1.59E+09	5.44E+07	2.33
D97	1.17E+09	5.41E+08	7.60	E127	1.66E+09	1.89E+08	4.14
G98 HA1	3.49E+08	7.81E+07	5.63	A128	7.61E+08	5.38E+08	8.90
G98 HA2	3.49E+08	3.07E+08	9.59	D129	1.05E+09	1.93E+08	5.16
F99	8.20E+08	6.85E+08	9.43	F130	1.21E+09	2.28E+08	5.21
I100	5.30E+08	3.11E+08	8.32	G132 HA1	1.10E+09	2.67E+08	5.83
S101	4.76E+08	1.50E+08	6.51	G132 HA2	1.10E+09	5.92E+07	2.90
A103	1.87E+09	2.10E+08	4.12	D133	7.38E+08	7.93E+08	10.23
E104	7.97E+08	2.07E+08	6.00	G134 HA1	8.55E+08	1.47E+08	5.00
L105	7.60E+08	1.36E+08	5.09	G134 HA2	8.55E+08	2.49E+08	6.30
R106	8.26E+08	8.23E+07	3.89	M135	1.24E+09	8.03E+08	8.63
F107	1.10E+09	5.44E+07	2.79	I136	4.17E+08	3.77E+08	9.68
V108	1.43E+08	8.70E+07	8.43	N137	3.75E+08	2.20E+08	8.32
M109	7.75E+08	1.29E+08	4.93	Y138	8.09E+08	2.21E+07	2.09
I110	1.98E+08	9.00E+07	7.55	E139	2.50E+09	3.64E+08	4.64
N111	6.45E+08	1.28E+08	5.34	E140	4.33E+08	1.27E+08	6.32
L112	4.95E+08	8.36E+07	4.96	F141	7.21E+08	9.40E+07	4.41
G113 HA1	1.09E+09	2.58E+08	5.76	V142	1.17E+09	6.72E+07	2.99
G113 HA2	1.09E+09	4.27E+08	7.12	W143	4.44E+08	9.81E+07	5.59
E114	1.18E+09	5.86E+08	7.82	M144	7.95E+08	1.95E+07	1.98
K115	1.27E+09	5.85E+08	7.59	S146	1.05E+09	3.92E+08	6.98
V116	1.69E+09	1.11E+09	8.67	Q147	9.82E+08	5.95E+08	8.42
T117	6.96E+08	3.65E+08	7.98	K148	3.11E+09	1.36E+09	7.44
D118	1.31E+09	8.83E+07	3.23				

C.7.2 $^3J_{HNHB}$

Residue	H_β	norml V_{HNB}	norml V_{HN}	$^3J_{HNHB}$	$^3J_{HNHB}$ with half Intensity	trans gauche to amide "N"	peak in HNCOHB	Stereo-assign
S2	HB#	5.33	55.85	2.63	(1.84,1.84)	dbl gauche		
E3	HB2	0.97	10.13	2.64		gauche	yes	SWAP
E3	HB3	2.17	10.13	4.03		trans		SWAP
L4	HB2	29.68	48.83	7.49		trans		
L4	HB3	6.14	48.83	3.04		gauche		
E6	HB#	17.88	118.10	3.35		avg	yes	
E7	HB2	17.23	80.57	4.03		trans		AS IS
E7	HB3	2.70	80.57	1.54		gauche	yes	AS IS
Q8	HB2	21.93	104.68	3.98		trans		
Q8	HB3	4.51	104.68	1.75		gauche		
I9	HB	2.82	104.37	1.38		gauche		
E11	HB#	15.97	144.04	2.84	(1.90,1.90)	dbl gauche		
F12	HB2	22.05	233.76	2.61		trans		
F12	HB3	7.03	233.76	1.46		gauche		
K13	HB2	2.40	28.08	2.49		avg	yes	
K13	HB3	2.55	28.08	2.56		avg		
D14	HB2	25.87	236.82	2.82		trans		AS IS
D14	HB3	4.30	236.82	1.13		gauche	yes	AS IS
F16	HB2	1.87	57.98	1.51		avg	yes	
F16	HB3	3.56	57.98	2.10		avg		
V17	HB	9.83	69.58	3.23		avg		
Q18	HB2	13.47	79.96	3.54		trans		
Q18	HB3	4.55	79.96	2.02		gauche		
F19	HB2	5.50	161.74	1.55		gauche		
F19	HB3	15.74	161.74	2.66		gauche		
D20	HB2	2.21	122.99	1.13		dG	yes	AS IS
D20	HB3	3.84	122.99	1.49		dG		AS IS
K21	HB#	43.75	132.75	5.12		avg		
E22	HB2	4.61	101.32	1.80		gauche		
E22	HB3	16.03	101.32	3.43		trans		
T24	HB	25.87	220.34	2.93		-	yes	
K26	HB3	15.02	187.07	2.41		-		
I27	HB	9.36	59.81	3.41		-		
R30	HB3	11.09	143.43	2.36		avg		
E31	HB2	2.86	236.82	0.92		dbl gauche		
E31	HB3	10.97	236.82	1.82		dbl gauche		
L32	HB2	2.32	49.44	1.83		avg		
L32	HB3	3.21	49.44	2.16		avg		
T34	HB	1.79	57.98	1.48		-		
L35	HB2	1.06	38.45	1.40		gauche	yes	SWAP
L35	HB3	3.51	38.45	2.57		trans		SWAP
M36	HB2	13.47	59.51	4.15		trans		
M26	HB3	1.50	59.51	1.34		gauche		

R37	HB3	12.81	67.44	3.78		trans		
T38	HB	9.18	115.05	2.40		-		
L39	HB2	8.82	99.18	2.54		gauche		
L39	HB3	21.16	99.18	4.02		trans		
Q41	HB2	2.57	86.06	1.46		gauche		
Q41	HB3	11.44	86.06	3.13		trans		
N42	HB2	0.57	44.56	0.95		gauche		
N42	HB3	3.22	44.56	2.28		trans		
T44	HB	17.94	189.21	2.62		-		
E45	HB#	14.13	59.20	4.27		avg	yes	
E47	HB2	23.25	187.07	3.02		trans		
E47	HB3	4.36	187.07	1.28		gauche		
L48	HB#	16.03	57.07	4.68		avg	yes	
Q49	HB2	21.28	135.19	3.42		trans		AS IS
Q49	HB3	5.03	135.19	1.63		gauche	yes	AS IS
D50	HB2	18.36	183.72	2.69		trans		
D50	HB3	9.42	183.72	1.91		gauche		
L51	HB2	21.40	87.89	4.32		trans		
L51	HB3	5.11	87.89	2.04		gauche		
E54	HB#	13.47	162.96	2.44	(1.71,1.71)	dbl gauche		
E56	HB#	19.49	84.84	4.19		avg		
N57	HB2	3.71	234.38	1.06		dbl gauche		
N57	HB3	8.64	234.38	1.62		dbl gauche		
N59	HB2	0.60	61.95	0.83		gauche		
N59	HB3	3.53	61.95	2.02		trans		
N60	HB2	1.15	39.06	1.44		gauche		
N60	HB3	3.21	39.06	2.43		trans		
Q62	HB2	14.66	57.98	4.41		trans		
L63	HB#	12.58	43.64	4.75		avg		
N64	HB2	4.51	172.73	1.36		gauche	yes	SWAP
N64	HB3	17.76	172.73	2.73		trans		SWAP
T66	HB	3.94	95.83	1.71		-		
E67	HB2	11.15	56.46	3.86		trans		
E67	HB3	2.81	56.46	1.89		gauche		
F68	HB#	2.84	47.61	2.07	(1.45,1.45)	dbl gauche		
C69	HB2	13.11	90.94	3.26		trans		
C69	HB3	4.58	90.94	1.90		gauche		
I71	HB	3.63	38.76	2.60		-		
M72	HB2	9.83	36.01	4.61		trans		
M72	HB3	1.29	36.01	1.59		gauche		
K74	HB2	6.79	86.67	2.38		avg		
K74	HB3	8.11	86.67	2.60		avg	yes	
Q75	HB#	7.93	63.78	3.02		avg	yes	
M76	HB2	3.90	99.18	1.67		G		
M76	HB3	13.95	99.18	3.22		T		
R77	HB#	15.50	67.14	4.20		avg		
E78	HB2	2.69	68.36	1.67		gauche	yes	SWAP
E78	HB3	13.71	68.36	3.89		trans		SWAP
T79	HB	10.61	95.83	2.84		-		
D80	HB2	3.24	96.44	1.54		gauche		

D80	HB3	9.18	96.44	2.63		trans		
T81	HB	6.26	61.34	2.72		-		
E82	HB2	2.08	79.96	1.36		gauche	yes	SWAP
E82	HB3	14.36	79.96	3.67		trans		SWAP
E83	HB#	11.68	81.48	3.25		avg		
E84	HB#	18.12	88.81	3.93		avg		
M85	HB2	9.30	88.20	2.77		avg		
M85	HB3	13.59	88.20	3.38		avg		
R86	HB2	6.32	29.63	4.02		avg		
R86	HB3	4.99	29.63	3.54		avg	yes	
E87	HB#	15.44	93.99	3.49		avg	yes	
K90	HB#	13.65	60.73	4.14		avg		
I91	HB	5.57	63.78	2.51		-		
F92	HB3	11.68	72.02	3.47		trans		
D93	HB2	0.75	41.50	1.13		dbl gauche	yes	AS IS
D93	HB3	1.29	41.50	1.48		dbl gauche		AS IS
R94	HB2	1.70	26.49	2.14		gauche		
R94	HB3	8.70	26.49	5.11		trans		
D95	HB2	4.73	213.01	1.25		gauche	yes	SWAP
D95	HB3	21.99	213.01	2.74		trans		SWAP
D97	HB2	4.18	165.10	1.34		gauche		
D97	HB3	15.85	165.10	2.64		trans		
F99	HB2	16.09	146.48	2.83		trans		
F99	HB3	6.08	146.48	1.72		gauche		
I100	HB	1.06	22.13	1.84		-		
S101	HB2	3.49	68.36	1.91		avg		
S101	HB3	6.68	68.36	2.66		avg		
E104	HB3	21.22	160.22	3.12		trans		
L105	HB2	3.18	70.19	1.80		dbl gauche	yes	AS IS
L105	HB3	3.20	70.19	1.80		dbl gauche		AS IS
R106	HB#	4.67	36.93	3.05	(2.13,2.13)	avg	yes	
F107	HB#	4.63	67.75	2.21	(1.55,1.55)	dbl gauche	yes	
V108	HB	2.71	42.11	2.15		-		
M109	HB2	12.04	80.87	3.32		trans		
M109	HB3	5.19	80.87	2.14		gauche		
I110	HB	3.58	32.96	2.81		-		
N111	HB2	5.45	127.87	1.74		avg		
N111	HB3	7.69	127.87	2.07		avg		
L112	HB2	11.62	88.50	3.11		trans		
L112	HB3	3.45	88.50	1.66		gauche		
E114	HB2	6.44	214.84	1.46		gauche		
E114	HB3	32.07	214.84	3.32		trans		
K115	HB2	2.97	78.13	1.64		gauche	yes	SWAP
K115	HB3	20.86	78.13	4.55		trans		SWAP
V116	HB	21.87	154.42	3.23		-	yes	
T117	HB	16.99	144.35	2.93		-		
D118	HB2	5.96	164.49	1.60		gauche		
D118	HB3	24.14	164.49	3.29		trans		
E119	HB2	20.33	112.00	3.69		trans		
E119	HB3	4.05	112.00	1.60		gauche		

E120	HB2	24.26	153.20	3.43		trans		
E120	HB3	8.29	153.20	1.97		gauche		
I121	HB	3.84	45.17	2.48		-		
D122	HB2	24.68	249.02	2.68		trans		
D122	HB3	8.88	249.02	1.59		gauche		
E123	HB#	20.38	85.45	4.27		avg	yes	
M124	HB2	13.29	131.53	2.71		trans		AS IS
M124	HB3	5.96	131.53	1.80		gauche	yes	AS IS
R126	HB2	18.48	64.09	4.75		trans		AS IS
R126	HB3	1.60	64.09	1.33		gauche	yes	AS IS
E127	HB#	21.52	135.50	3.43		avg	yes	
D129	HB2	1.11	54.63	1.20		dbl gauche	yes	AS IS
D129	HB3	1.39	54.63	1.34		dbl gauche		AS IS
F130	HB2	7.33	174.56	1.73		gauche		
F130	HB3	22.05	174.56	3.04		trans		
D131	HB2	2.84	116.88	1.31		gauche	yes	SWAP
D131	HB3	14.31	116.88	2.99		trans		SWAP
D133	HB2	3.46	153.81	1.26		gauche	yes	SWAP
D133	HB3	15.14	153.81	2.67		trans		SWAP
M135	HB2	15.08	92.47	3.48		trans		
M135	HB3	3.75	92.47	1.70		gauche		
I136	HB	2.35	36.01	2.16		-		
N137	HB#	8.82	39.37	4.13		avg		
Y138	HB2	0.76	32.04	1.30		gauche	yes	SWAP
Y138	HB3	5.08	32.04	3.43		trans		SWAP
E139	HB2	29.92	133.97	4.12		trans		
E139	HB3	6.62	133.97	1.88		gauche		
E140	HB2	14.13	94.91	3.32		trans		
E140	HB3	2.14	94.91	1.26		gauche		
F141	HB2	0.56	34.48	1.07		dbl gauche	yes	AS IS
F141	HB3	1.86	34.48	1.96		dbl gauche		AS IS
V142	HB	4.79	77.82	2.10		-		
W143	HB2	0.96	88.20	0.88		dbl gauche	yes	AS IS
W143	HB3	3.18	88.20	1.60		dbl gauche		AS IS
M144	HB#	4.67	50.05	2.60	(1.82,1.82)	dbl gauche		
I145	HB	4.77	40.59	2.93		-		
S146	HB#	18.95	157.17	2.97	(2.08,2.08)	avg	yes	
Q147	HB2	5.44	107.73	1.90		gauche	yes	SWAP
Q147	HB3	23.13	107.73	4.03		trans		SWAP
K148	HB2	18.30	126.34	3.27		gauche		
K148	HB3	44.05	126.34	5.29		trans		

APPENDIX D

D.1 Data processing macros for 3D HCCH-COSY and 3D N-NOESY spectra for apoN androcam

D.1.1 HCCH-COSY

File name : "fid.com"

```
#!/bin/csh

var2pipe -in ./fid \
 -noaswap -aqORD 1 \
  -xN              2048  -yN               320  -zN                88  \
  -xT              1024  -yT               160  -zT                44  \
  -xMODE        Complex  -yMODE         Complex  -zMODE         Complex  \
  -xSW        11990.408  -ySW          8798.944  -zSW          4022.728  \
  -xOBS         799.872  -yOBS          799.872  -zOBS          201.133  \
  -xCAR           4.773  -yCAR            4.773  -zCAR           35.033  \
  -xLAB              HN  -yLAB              H1  -zLAB              C13  \
  -ndim               3  -aq2D          States                         \
  -out ./data/test%03d.fid -verb -ov

sleep 5
```

File name : "nmrproc.com"

```
#!/bin/csh

#
# 3D States-Mode HN-Detected Processing.

xyz2pipe -in data/test%03d.fid -x -verb \
| nmrPipe -fn POLY -time                              \
| nmrPipe  -fn SP -off 0.5 -end 0.98 -pow 2 -c 0.5    \
| nmrPipe  -fn ZF -auto                               \
| nmrPipe  -fn FT                                     \
| nmrPipe  -fn PS -p0 79.5  -p1 43.0 -di              \
| nmrPipe  -fn EXT -x1 -1.0ppm    -xn 6.5ppm   -sw    \
| nmrPipe  -fn TP                                     \
| nmrPipe  -fn SP -off 0.5 -end 0.98 -pow 1 -c 1.0    \
| nmrPipe  -fn ZF -auto                               \
| nmrPipe  -fn FT                                     \
| nmrPipe  -fn PS -p0 -46.2 -p1 180 -di               \
| pipe2xyz -out ft/test%03d.ft2 -y

xyz2pipe -in ft/test%03d.ft2 -z -verb                \
| nmrPipe -fn MAC -macro hamm51.M -var fst 5 -verb    \
| nmrPipe -fn EM  -lb 10.0 -c 1.0                     \
| nmrPipe  -fn ZF -auto                               \
| nmrPipe -fn ZF -auto                                \
| nmrPipe  -fn FT                                     \
| nmrPipe  -fn PS -p0 -54.0 -p1 180.0 -di             \
| nmrPipe -fn POLY -auto                              \
| pipe2xyz -out ft/test%03d.ft3 -z
```

D.1.2 N-NOESY (gradient selection of pulses, sensitivity enhancement)

File name : "fid.com"

```
#!/bin/csh

var2pipe -in ./fid \
 -noaswap -aqORD 1 \
  -xN              2048  -yN               480  -zN               120  \
  -xT              1024  -yT               240  -zT                60  \
  -xMODE        Complex  -yMODE         Complex  -zMODE       Rance-Kay  \
  -xSW        11990.408  -ySW          9990.000  -zSW          1337.480  \
  -xOBS         799.872  -yOBS          799.872  -zOBS           81.059  \
  -xCAR           4.773  -yCAR             4.773  -zCAR          117.212  \
  -xLAB              HN  -yLAB               H1  -zLAB              N15  \
  -ndim               3  -aq2D           States                          \
  -out ./data/test%03d.fid -verb -ov

sleep 5
```

File name : "nmrproc.com"

```
#!/bin/csh

#
# 3D States-Mode HN-Detected Processing.

xyz2pipe -in data/test%03d.fid -x -verb \
| nmrPipe  -fn SOL                                  \
| nmrPipe  -fn SP -off 0.5 -end 0.98 -pow 2 -c 0.5  \
| nmrPipe  -fn ZF -auto                             \
| nmrPipe  -fn FT                                   \
| nmrPipe  -fn PS -p0 188.5  -p1  -14.0 -di         \
| nmrPipe  -fn EXT -x1 5.5ppm -xn 11.0ppm -sw       \
| nmrPipe  -fn TP                                   \
| nmrPipe  -fn SP -off 0.5 -end 0.98 -pow 1 -c 1.0  \
| nmrPipe  -fn ZF -auto                             \
| nmrPipe  -fn FT                                   \
| nmrPipe  -fn PS -p0 -46.4 -p1 180 -di             \
| pipe2xyz -out ft/test%03d.ft2 -y

xyz2pipe -in ft/test%03d.ft2 -z -verb              \
| nmrPipe -fn LP -fb -pred 60 -ord 24 -fixMode 1 -verb    \
| nmrPipe -fn MAC -macro hamm51.M -var fst 5 -verb        \
| nmrPipe -fn EM -lb 10.0 -c 1.0                          \
| nmrPipe -fn SP  -off 0.5 -end 0.98 -pow 1 -c 1.0  \
| nmrPipe  -fn ZF -auto                             \
| nmrPipe  -fn ZF -auto                             \
| nmrPipe  -fn FT                                   \
| nmrPipe  -fn PS -p0 -90.0 -p1 180.0 -di           \
| pipe2xyz -out ft/test%03d.ft3      -z
```

D.2 psf files for including Ca^{+2} ion for holo and apoN androcam

File Name : "2ca.psf"

```
data_cns_mtf
_cns_mtf.title
; FILENAME="generate_seq.mtf"
  file toppar/protein.link
  this is a macro to define standard protein peptide bonds
  and termini to generate a protein sequence.
  DATE:11-Oct-2008  16:59:54       created by user: mkjoshi
  VERSION:1.21 ;
loop_
_cns_mtf_atom.id
_cns_mtf_atom.segment_id
_cns_mtf_atom.residue_id
_cns_mtf_atom.residue_name
_cns_mtf_atom.atom_name
_cns_mtf_atom.chemical_type
_cns_mtf_atom.charge
_cns_mtf_atom.atom_mass
1 ' ' '149' 'CA2' 'CA+2' 'CA+2' 2.00000 40.0800
2 ' ' '150' 'CA2' 'CA+2' 'CA+2' 2.00000 40.0800
-1 ' ' ' ' ' ' ' ' ' ' ' -1.00000 -1.00000
loop_
_cns_mtf_bond.id[1]
_cns_mtf_bond.id[2]
-1 -1
loop_
_cns_mtf_angle.id[1]
_cns_mtf_angle.id[2]
_cns_mtf_angle.id[3]
-1 -1 -1
loop_
_cns_mtf_dihedral.id[1]
_cns_mtf_dihedral.id[2]
_cns_mtf_dihedral.id[3]
_cns_mtf_dihedral.id[4]
-1 -1 -1 -1
loop_
_cns_mtf_improper.id[1]
_cns_mtf_improper.id[2]
_cns_mtf_improper.id[3]
_cns_mtf_improper.id[4]
-1 -1 -1 -1
loop_
_cns_mtf_explicit_nonbonded_exclusion.inb
-1
loop_
_cns_mtf_explicit_nonbonded_exclusion.iblo
0
0
-1
loop_
_cns_mtf_group_linked_list.first_atom_id
0
1
-1
```

File Name : "3ca.psf"

```
data_cns_mtf
_cns_mtf.title;
FILENAME="generate_seq.mtf"
  file toppar/protein.link  this is a macro to define standard protein peptide bonds and
termini to generate a protein sequence.
  DATE:21-Oct-2008  10:53:56  created by user: mkjoshi  VERSION:1.21;
loop_
_cns_mtf_atom.id
_cns_mtf_atom.segment_id
_cns_mtf_atom.residue_id
_cns_mtf_atom.residue_name
_cns_mtf_atom.atom_name
_cns_mtf_atom.chemical_type
_cns_mtf_atom.charge
_cns_mtf_atom.atom_mass
1 ' ' '149' 'CA2' 'CA+2' 'CA+2' 2.00000 40.0800
2 ' ' '150' 'CA2' 'CA+2' 'CA+2' 2.00000 40.0800
3 ' ' '151' 'CA2' 'CA+2' 'CA+2' 2.00000 40.0800
-1 ' ' ' ' ' ' ' ' ' ' ' -1.00000 -1.00000
loop_
_cns_mtf_bond.id[1]
_cns_mtf_bond.id[2]
-1 -1
loop_
_cns_mtf_angle.id[1]
_cns_mtf_angle.id[2]
_cns_mtf_angle.id[3]
-1 -1 -1
loop_
_cns_mtf_dihedral.id[1]
_cns_mtf_dihedral.id[2]
_cns_mtf_dihedral.id[3]
_cns_mtf_dihedral.id[4]
-1 -1 -1 -1
loop_
_cns_mtf_improper.id[1]
_cns_mtf_improper.id[2]
_cns_mtf_improper.id[3]
_cns_mtf_improper.id[4]
-1 -1 -1 -1
loop_
_cns_mtf_explicit_nonbonded_exclusion.inb
-1
loop_
_cns_mtf_explicit_nonbonded_exclusion.iblo
0
0
0
-1
loop_
_cns_mtf_group_linked_list.first_atom_id
0
1
2
-1
```

D.3 parameter file for including Ca^{+2} ion (holo and apoN androcam)

File Name : "Ion.param"

```
remarks file toppar/ion.param
remarks nonbonded parameters for common ions
remarks new parameters derived from literature for single atom species
remarks PDA 02/09/99
set echo=off end
checkversion 1.1
BOND  OUF SUF          1480.000    1.475
BOND  OHF PHF          1489.209    1.485
BOND  OWF W            1489.209    1.730
ANGLE OUF SUF OUF       337.400  109.400
ANGLE OHF PHF OHF      1337.074  119.600
ANGLE OWF W   OWF      1337.074  119.600
!                 eps     sigma       eps(1:4) sigma(1:4)
!               (kcal/mol)  (A)       (kcal/mol)   (A)
!                 ------------------------------------
{- neutral radii -}{- from: Teatum, Gschneider & Waber. (1960) Compilation of calculated
data useful in predicting metallurgical behaviour of the elements in binary alloy
systems, LE-2345, Los Alamos Scientific Laboratory -}
NONBonded  LI     0.01  2.783     0.01  2.783
NONBonded   F     0.01  2.619     0.01  2.619
NONBonded  NA     0.01  3.405     0.01  3.405
NONBonded  MG     0.01  2.854     0.01  2.854
NONBonded  AL     0.01  2.552     0.01  2.552
NONBonded  CL     0.01  3.118     0.01  3.118
NONBonded   K     0.01  4.234     0.01  4.234
NONBonded  AR     0.01  3.200     0.01  3.200
NONBonded  CA     0.01  3.517     0.01  3.517
NONBonded   V     0.01  2.398     0.01  2.398
NONBonded  CR     0.01  2.284     0.01  2.284
NONBonded  MN     0.01  2.252     0.01  2.252
NONBonded  FE     0.01  2.270     0.01  2.270
NONBonded  NI     0.01  2.220     0.01  2.220
NONBonded  CO     0.01  2.231     0.01  2.231
NONBonded  CU     0.01  2.277     0.01  2.277
NONBonded  ZN     0.01  2.484     0.01  2.484
NONBonded  AS     0.01  3.296     0.01  3.296
NONBonded  BR     0.01  3.296     0.01  3.296
NONBonded  KR     0.01  3.400     0.01  3.400
NONBonded  SR     0.01  3.833     0.01  3.833
NONBonded  MO     0.01  2.495     0.01  2.495
NONBonded  AG     0.01  2.575     0.01  2.575
NONBonded  CD     0.01  2.794     0.01  2.794
NONBonded   I     0.01  3.528     0.01  3.528
NONBonded  XE     0.01  3.564     0.01  3.564
NONBonded  CS     0.01  4.866     0.01  4.866
NONBonded  HO     0.01  3.147     0.01  3.147
NONBonded  YB     0.01  3.100     0.01  3.100
NONBonded  OS     0.01  2.411     0.01  2.411
NONBonded  IR     0.01  2.418     0.01  2.418
NONBonded  PT     0.01  2.471     0.01  2.471
NONBonded  AU     0.01  2.569     0.01  2.569
NONBonded  HG     0.01  2.803     0.01  2.803
NONBonded  PB     0.01  3.118     0.01  3.118
NONBonded   U     0.01  2.780     0.01  2.780
{- ionic radii -}{- from: Shannon (1976) Revised effective ionic radii in halides and
chalcogenides, Acta Cryst. A32, 751. -}
NONBonded  LI+1   0.01  1.604     0.01  1.604
NONBonded   F-1   0.01  2.120     0.01  2.120
NONBonded  NA+1   0.01  2.067     0.01  2.067
NONBonded  MG+2   0.01  1.532     0.01  1.532
NONBonded  AL+3   0.01  1.203     0.01  1.203
NONBonded  CL-1   0.01  2.976     0.01  2.976
NONBonded   K+1   0.01  2.708     0.01  2.708
NONBonded  CA+2   0.01  2.031     0.01  2.031
NONBonded   V+2   0.01  1.657     0.01  1.657
```

```
NONBonded   V+3    0.01  1.390     0.01  1.390
NONBonded  CR+2    0.01  1.550     0.01  1.550
NONBonded  CR+3    0.01  1.345     0.01  1.345
NONBonded  MN+2    0.01  1.443     0.01  1.443
NONBonded  MN+3    0.01  1.283     0.01  1.283
NONBonded  FE+2    0.01  1.336     0.01  1.336
NONBonded  FE+3    0.01  1.229     0.01  1.229
NONBonded  NI+2    0.01  1.479     0.01  1.479
NONBonded  CO+2    0.01  1.408     0.01  1.408
NONBonded  CO+3    0.01  1.221     0.01  1.221
NONBonded  CU+1    0.01  1.621     0.01  1.621
NONBonded  CU+2    0.01  1.550     0.01  1.550
NONBonded  ZN+2    0.01  1.568     0.01  1.568
NONBonded  BR-1    0.01  3.243     0.01  3.243
NONBonded  SR+2    0.01  2.352     0.01  2.352
NONBonded  MO+3    0.01  1.479     0.01  1.479
NONBonded  AG+1    0.01  2.299     0.01  2.299
NONBonded  CD+2    0.01  1.942     0.01  1.942
NONBonded   I-1    0.01  3.671     0.01  3.671
NONBonded  CS+1    0.01  3.225     0.01  3.225
NONBonded  HO+3    0.01  1.855     0.01  1.855
NONBonded  YB+2    0.01  2.067     0.01  2.067
NONBonded  YB+3    0.01  1.796     0.01  1.796
NONBonded  OS+4    0.01  1.372     0.01  1.372
NONBonded  IR+3    0.01  1.461     0.01  1.461
NONBonded  PT+2    0.01  1.675     0.01  1.675
NONBonded  AU+1    0.01  2.691     0.01  2.691
NONBonded  AU+3    0.01  1.764     0.01  1.764
NONBonded  HG+1    0.01  2.370     0.01  2.370
NONBonded  HG+2    0.01  2.067     0.01  2.067
NONBonded  PB+2    0.01  2.370     0.01  2.370
NONBonded   U+3    0.01  2.076     0.01  2.076
NONBonded   U+4    0.01  1.835     0.01  1.835
{- multi-atom species -}
{- source of parameters unknown, therefore possibly in error -}
NONBonded   SUF    0.01  3.368     0.01  3.368
NONBonded   OUF    0.01  2.851     0.01  2.851
NONBonded   PHF    0.01  3.385     0.01  3.385
NONBonded   OHF    0.01  2.729     0.01  2.729
NONBonded     W    0.01  2.305     0.01  2.305
NONBonded   OWF    0.01  2.729     0.01  2.729
set echo=on end
```

D.4 User input paramters for ARIA run (holo and apoN androcam)

File Name : "run.cns"

```
!$Revision: 2.10 $
!$Date: 2002/07/23 16:19:27 $
!$RCSfile: run.cns,v $

module(
spectrum;
iteration;
filenames;
spectra;
data;
iterations;
saprotocol;
refine;
relax;
toppar;
analysis;
)
{+ file: run.cns +}
{+ description: The file run.cns contains all necessary information to run ARIA.
ARIA automatically sets the default values.
Please change the values for the mixing time, rotation
correlation time and spectrometer frequency for the spin diffusion correction.
For the large CNS log files, you should use a temporary directory. version 1.2 +}
{+ authors: Jens Linge, Michael Nilges +}
set message off echo off end
! Please cite the following references when using this protocol:
{+ reference: M. Nilges (1995) Calculation of protein structures with ambiguous distance
restraints. Automated assignment of ambiguous NOE crosspeaks and disulphide
connectivities. J. Mol. Biol. 245, 645-660 +}
{+ reference: M. Nilges and Sean O'Donoghue (1998) Ambiguous NOEs and automated NOE
assignment. Prog. NMR Spect. 32, 107-139 +}
{+ reference: J.P. Linge and M. Nilges (1999) Influence of non-bonded parameters on the
quality of NMR structures: a new force-field for NMR structure calculation. J. Biomol.
NMR 13, 51-59 +}
{+ reference:        J.P. Linge (2001) New methods for automated NOE assignment and NMR
structure calculation. Book on Demand Verlag        ISBN: 383111482X  +}
{+ reference:        J.P. Linge, S.I. O'Donoghue and M. Nilges (2001). Assigning
Ambiguous NOEs with ARIA.
       Methods in Enzymology, 339, 71-90. +}
{- Guidelines for using this file:
   - all strings must be quoted by double-quotes
   - logical variables (true/false) are not quoted
   - do not remove any evaluate statements from the file
   - pathnames should not exceed 80 characters -}
{- begin block parameter definition -} define(
{====================== filenames ======================}
{*  the name of your current project *}
{===>} fileroot="20100423";
{* RUN directory *}
{*  the absolute path of your current run, e.g. /home/linge/werner/run3 *}
{===>} run_dir="/data/mkjoshi/apoN_ACaM/aria1.2/20100423/run4";
{* molecular topology files (mtf) *}
{* will be generated by ARIA from the sequence / add other mtf files here *}
{===>} structure="20100423.psf";
{===>} structure_2="2ca.psf";
{===>} structure_3="";
{===>} structure_4="";
{===>} structure_5="";
{* PDB or sequence (3-letter code) file*}
{+ choice: "PDB" "sequence" +}
{===>} pdb_or_sequence="sequence";
{===>} prot_coor_1="acam.seq";

{* new segment id (segid) *}
```

```
{* will appear in the pdb files, must have 4 digits, leave it blank if you don't want one
*}
{===>} prot_segid_1="";

{* Atomname nomenclature *}
{* set true if you have IUPAC (e.g. LEU HB2 and HB3 and not HB2 and HB1) data (e.g. from
XEASY) *}
{+ choice: true false +}
{===>} xplortodiana=true;

{* ARIA directory *}
{*  the absolute path of the ARIA program files *}
{===>} aria_dir="/divsite/opt.BIOC/aria1.2/redhat9";

{* Logfile directory *}
{* specify a directory for the large CNS log files *}
{===>} temptrash_dir="/data/mkjoshi/apoN_ACaM/aria1.2/20100423/run4";

{============================ disulphide bonds ==============================}
{* number of disulphide bridges *}
{* set to zero if none are present *}
{===>} ss_bridge=0;

{* unambiguous or ambiguous disulphide bridges *}
{* default: unambiguous *}
{+ choice: "unambiguous" "ambiguous" +}
{===>} ss_ambigunambig="unambiguous";

{* repeat the four lines for each disulfide bond *}
{* first pair of entries are the resid and segid of the first cysteine *}
{* second pair of entries are the resid id and segid of the second cysteine *}
{===>}
 ss_i_resid_1=11;
 ss_i_segid_1="";
 ss_j_resid_1=22;
 ss_j_segid_1="";

 ss_i_resid_2=33;
 ss_i_segid_2="";
 ss_j_resid_2=44;
 ss_j_segid_2="";

 ss_i_resid_3=55;
 ss_i_segid_3="";
 ss_j_resid_3=66;
 ss_j_segid_3="";

 ss_i_resid_4=77;
 ss_i_segid_4="";
 ss_j_resid_4=88;
 ss_j_segid_4="";

 ss_i_resid_5=99;
 ss_i_segid_5="";
 ss_j_resid_5=01;
 ss_j_segid_5="";

 {<===}

{============================ histidine patches ==============================}
{* Patch to change doubly protonated HIS to singly protonated histidine (HD1) *}
{* just give the residue number of the histidines for the HISD patch, set them to zero if
you don't want them *}
{+ table: rows=2 "residue" "segid" cols=10 "1" "2" "3" "4" "5" "6" "7" "8" "9" "10" +}
{===>} hisd_resid_1=0;
{===>} hisd_resid_2=0;
{===>} hisd_resid_3=0;
{===>} hisd_resid_4=0;
{===>} hisd_resid_5=0;
{===>} hisd_resid_6=0;
{===>} hisd_resid_7=0;
```

```
{===>} hisd_resid_8=0;
{===>} hisd_resid_9=0;
{===>} hisd_resid_10=0;
{===>} hisd_segid_1="";
{===>} hisd_segid_2="";
{===>} hisd_segid_3="";
{===>} hisd_segid_4="";
{===>} hisd_segid_5="";
{===>} hisd_segid_6="";
{===>} hisd_segid_7="";
{===>} hisd_segid_8="";
{===>} hisd_segid_9="";
{===>} hisd_segid_10="";

{* Patch to change doubly protonated HIS to singly protonated histidine (HE2) *}
{* just give the residue number of the histidines for the HISE patch, set them to zero if
you don't want them *}
{+ table: rows=2 "residue" "segid" cols=10 "1" "2" "3" "4" "5" "6" "7" "8" "9" "10" +}
{===>} hise_resid_1=0;
{===>} hise_resid_2=0;
{===>} hise_resid_3=0;
{===>} hise_resid_4=0;
{===>} hise_resid_5=0;
{===>} hise_resid_6=0;
{===>} hise_resid_7=0;
{===>} hise_resid_8=0;
{===>} hise_resid_9=0;
{===>} hise_resid_10=0;
{===>} hise_segid_1="";
{===>} hise_segid_2="";
{===>} hise_segid_3="";
{===>} hise_segid_4="";
{===>} hise_segid_5="";
{===>} hise_segid_6="";
{===>} hise_segid_7="";
{===>} hise_segid_8="";
{===>} hise_segid_9="";
{===>} hise_segid_10="";

{============================ Distance restraints  ========================}
{* Do you want to use hbond restraints? *}
{+ choice: true false +}
{===>} hbonds_on=true;

{* energy constants *}
{+ table: rows=3 "unambig" "ambig" "hbonds" cols=5 "firstIteration" "hot" "cool1_ini"
"cool1_fin" "cool2" +}

{===>} unamb_firstit=0;
{===>} unamb_hot=10;
{===>} unamb_cool1_ini=10;
{===>} unamb_cool1_fin=50;
{===>} unamb_cool2=50;
{===>} amb_firstit=0;
{===>} amb_hot=10;
{===>} amb_cool1_ini=10;
{===>} amb_cool1_fin=50;
{===>} amb_cool2=50;
{===>} hbond_firstit=0;
{===>} hbond_hot=10;
{===>} hbond_cool1_ini=10;
{===>} hbond_cool1_fin=50;
{===>} hbond_cool2=50;

{* potential shape *}
{+ table: rows=4 "mRswitch" "rswitch" "mAsymptote" "asymptote" cols=3 "hot" "cool1"
"cool2" +}
{===>} mrswi_hot=0.5;
{===>} mrswi_cool1=0.5;
{===>} mrswi_cool2=0.5;
{===>} rswi_hot=0.5;
```

```
{===>} rswi_cool1=0.5;
{===>} rswi_cool2=0.5;
{===>} masy_hot=-1.0;
{===>} masy_cool1=-1.0;
{===>} masy_cool2=-0.1;
{===>} asy_hot=1.0;
{===>} asy_cool1=1.0;
{===>} asy_cool2=0.1;

{========================== dihedrals ============================}
{* energy constants *}
{+ table: rows=1 "dihedrals" cols=4 "use?" "hot" "cool1" "cool2" +}

{+ choice: true false +}
{===>} dihedrals_on=true;
{===>} dihedrals_hot=5;
{===>} dihedrals_cool1=25;
{===>} dihedrals_cool2=200;

{========================== Karplus coupling restraints ==================}

{* Karplus coefficients *}
{+ table: rows=5 "class1" "class2" "class3" "class4" "class5"
cols=8 "use?" "A" "B" "C" "delta" "E(hot)" "E(cool1)" "E(cool2)" +}
{+ choice: true false +}
{===>} c1_on=true;
{===>} c1_karplusa=6.98;
{===>} c1_karplusb=-1.38;
{===>} c1_karplusc=1.72;
{===>} c1_karplusd=-180.0;
{===>} c1_hot=0.0;
{===>} c1_cool1=0.2;
{===>} c1_cool2=1.0;
{+ choice: true false +}
{===>} c2_on=false;
{===>} c2_karplusa=6.98;
{===>} c2_karplusb=-1.38;
{===>} c2_karplusc=1.72;
{===>} c2_karplusd=-120.0;
{===>} c2_hot=0.0;
{===>} c2_cool1=0.2;
{===>} c2_cool2=1.0;
{+ choice: true false +}
{===>} c3_on=false;
{===>} c3_karplusa=6.98;
{===>} c3_karplusb=-1.38;
{===>} c3_karplusc=1.72;
{===>} c3_karplusd=-120.0;
{===>} c3_hot=0.0;
{===>} c3_cool1=0.2;
{===>} c3_cool2=1.0;
{+ choice: true false +}
{===>} c4_on=false;
{===>} c4_karplusa=6.98;
{===>} c4_karplusb=-1.38;
{===>} c4_karplusc=1.72;
{===>} c4_karplusd=-120.0;
{===>} c4_hot=0.0;
{===>} c4_cool1=0.2;
{===>} c4_cool2=1.0;
{+ choice: true false +}
{===>} c5_on=false;
{===>} c5_karplusa=6.98;
{===>} c5_karplusb=-1.38;
{===>} c5_karplusc=1.72;
{===>} c5_karplusd=-120.0;
{===>} c5_hot=0.0;
{===>} c5_cool1=0.2;
{===>} c5_cool2=1.0;

{========================== residual dipolar couplings ====================}
```

```
{* Parameters *}
{+ table: rows=5 "class1" "class2" "class3" "class4" "class5"
          cols=19 "type" "firstIt" "E(hot)" "E(cool1)" "E(cool2)" "R" "D" "ini_bor_hot"
"fin_bor_hot"
 "ini_bor_cool1"  "fin_bor_cool1" "ini_bor_cool2" "fin_bor_cool2" "ini_cen_hot"
"fin_cen_hot"
 "ini_cen_cool1" "fin_cen_cool1" "ini_cen_cool2" "fin_cen_cool2"  +}
{+ choice: "NO" "SANI" "VANGLE" +}
{===>} rdc1_choice="NO";
{===>} rdc1_firstIt=0;
{===>} rdc1_hot=0.0;
{===>} rdc1_cool1=0.2;
{===>} rdc1_cool2=1.0;
{===>} rdc1_r=0.4;
{===>} rdc1_d=8.0;
{===>} ini_bor_hot_1=0.1;
{===>} fin_bor_hot_1=40.0;
{===>} ini_bor_cool1_1=40.0;
{===>} fin_bor_cool1_1=40.0;
{===>} ini_bor_cool2_1=40.0;
{===>} fin_bor_cool2_1=40.0;
{===>} ini_cen_hot_1=0.1;
{===>} fin_cen_hot_1=0.1;
{===>} ini_cen_cool1_1=10.0;
{===>} fin_cen_cool1_1=10.0;
{===>} ini_cen_cool2_1=10.0;
{===>} fin_cen_cool2_1=10.0;

{+ choice: "NO" "SANI" "VANGLE" +}
{===>} rdc2_choice="NO";
{===>} rdc2_firstIt=0;
{===>} rdc2_hot=0.0;
{===>} rdc2_cool1=0.2;
{===>} rdc2_cool2=1.0;
{===>} rdc2_r=0.4;
{===>} rdc2_d=8.0;
{===>} ini_bor_hot_2=0.1;
{===>} fin_bor_hot_2=40.0;
{===>} ini_bor_cool1_2=40.0;
{===>} fin_bor_cool1_2=40.0;
{===>} ini_bor_cool2_2=40.0;
{===>} fin_bor_cool2_2=40.0;
{===>} ini_cen_hot_2=0.1;
{===>} fin_cen_hot_2=0.1;
{===>} ini_cen_cool1_2=10.0;
{===>} fin_cen_cool1_2=10.0;
{===>} ini_cen_cool2_2=10.0;
{===>} fin_cen_cool2_2=10.0;

{+ choice: "NO" "SANI" "VANGLE" +}
{===>} rdc3_choice="NO";
{===>} rdc3_firstIt=0;
{===>} rdc3_hot=0.0;
{===>} rdc3_cool1=0.2;
{===>} rdc3_cool2=1.0;
{===>} rdc3_r=0.4;
{===>} rdc3_d=8.0;
{===>} ini_bor_hot_3=0.1;
{===>} fin_bor_hot_3=40.0;
{===>} ini_bor_cool1_3=40.0;
{===>} fin_bor_cool1_3=40.0;
{===>} ini_bor_cool2_3=40.0;
{===>} fin_bor_cool2_3=40.0;
{===>} ini_cen_hot_3=0.1;
{===>} fin_cen_hot_3=0.1;
{===>} ini_cen_cool1_3=10.0;
{===>} fin_cen_cool1_3=10.0;
{===>} ini_cen_cool2_3=10.0;
{===>} fin_cen_cool2_3=10.0;
```

```
{+ choice: "NO" "SANI" "VANGLE" +}
{===>} rdc4_choice="NO";
{===>} rdc4_firstIt=0;
{===>} rdc4_hot=0.0;
{===>} rdc4_cool1=0.2;
{===>} rdc4_cool2=1.0;
{===>} rdc4_r=0.4;
{===>} rdc4_d=8.0;
{===>} ini_bor_hot_4=0.1;
{===>} fin_bor_hot_4=40.0;
{===>} ini_bor_cool1_4=40.0;
{===>} fin_bor_cool1_4=40.0;
{===>} ini_bor_cool2_4=40.0;
{===>} fin_bor_cool2_4=40.0;
{===>} ini_cen_hot_4=0.1;
{===>} fin_cen_hot_4=0.1;
{===>} ini_cen_cool1_4=10.0;
{===>} fin_cen_cool1_4=10.0;
{===>} ini_cen_cool2_4=10.0;
{===>} fin_cen_cool2_4=10.0;

{+ choice: "NO" "SANI" "VANGLE" +}
{===>} rdc5_choice="NO";
{===>} rdc5_firstIt=0;
{===>} rdc5_hot=0.0;
{===>} rdc5_cool1=0.2;
{===>} rdc5_cool2=1.0;
{===>} rdc5_r=0.4;
{===>} rdc5_d=8.0;
{===>} ini_bor_hot_5=0.1;
{===>} fin_bor_hot_5=40.0;
{===>} ini_bor_cool1_5=40.0;
{===>} fin_bor_cool1_5=40.0;
{===>} ini_bor_cool2_5=40.0;
{===>} fin_bor_cool2_5=40.0;
{===>} ini_cen_hot_5=0.1;
{===>} fin_cen_hot_5=0.1;
{===>} ini_cen_cool1_5=10.0;
{===>} fin_cen_cool1_5=10.0;
{===>} ini_cen_cool2_5=10.0;
{===>} fin_cen_cool2_5=10.0;

{========================== CSI restraints ==============================}
{* Do you want to use CSI derived hbond restraints? *}
{* uses the files generated by the program CSI *}
{+ choice: true false +}
{===>} hbondscsi_on=false;
{* Do you want to use CSI derived dihedral restraints? *}
{+ choice: true false +}
{===>} dihedralscsi_on=false;

{========================== TALOS restraints ============================}
{* Do you want to use TALOS derived dihedral restraints? *}
{+ choice: true false +}
{===>} dihedralstalos_on=false;

{====================== topology and parameter files ====================}

{* topology file *}
{===>} prot_top="topallhdg5.3.pro";

{* linkage file *}
{===>} prot_link="topallhdg5.3.pep";

{* energy parameter file *}
{===>} prot_par_1="parallhdg5.3.pro";
{===>} prot_par_2="dna-rna-allatom.param";
{===>} prot_par_3="ion.param";
{===>} prot_par_4="";
{===>} prot_par_5="";
```

```
{* type of non-bonded parameters *}
{* specify the type of non-bonded interaction *}
{+ choice: "PROLSQ" "PARMALLH6" "PARALLHDG" "OPLSX" +}
{===>} par_nonbonded="PROLSQ";

{* Do you want to include dihedral angle energy terms? *}
{+ choice: true false +}
{===>} dihedflag=true;

{* Do you want to use floating chirality assignment (swapping)? *}
{+ choice: true false +}
{===>} swapflag=false;

{====================== spectra parameters ================}


{* parameters *}
{+ table: rows=19
   "spectra names"  "qshifts"  "qcalib"
   "qrelax" "Tmix" "Tcorrel" "frequency"
   "qerrset" "errmod" "err0" "err1" "err2" "err3" "qexclude" "qmove" "het1window"
"pro1window" "het2window" "pro2window"
    cols = 5 "1" "2" "3" "4" "5" +}

{===>} aspectrum_1="C_NOE";
{===>} aspectrum_2="N_NOE";
{===>} aspectrum_3="Arom_NOE";
{===>} aspectrum_4="";
{===>} aspectrum_5="";

{+ choice: true false +}
{===>} qshifts_1=true;
{+ choice: true false +}
{===>} qshifts_2=true;
{+ choice: true false +}
{===>} qshifts_3=true;
{+ choice: true false +}
{===>} qshifts_4=true;
{+ choice: true false +}
{===>} qshifts_5=true;

{+ choice: true false +}
{===>} qcalib_1=true;
{+ choice: true false +}
{===>} qcalib_2=true;
{+ choice: true false +}
{===>} qcalib_3=true;
{+ choice: true false +}
{===>} qcalib_4=true;
{+ choice: true false +}
{===>} qcalib_5=true;

{+ choice: true false +}
{===>} qrelax_1=true;
{+ choice: true false +}
{===>} qrelax_2=true;
{+ choice: true false +}
{===>} qrelax_3=true;
{+ choice: true false +}
{===>} qrelax_4=true;
{+ choice: true false +}
{===>} qrelax_5=true;

{===>} tmix_1=0.080;
{===>} tmix_2=0.080;
{===>} tmix_3=0.080;
{===>} tmix_4=0.080;
{===>} tmix_5=0.080;

{===>} tcorrel_1=8E-9;
{===>} tcorrel_2=8E-9;
```

```
{===>} tcorrel_3=8E-9;
{===>} tcorrel_4=8E-9;
{===>} tcorrel_5=8E-9;

{===>} frequency_1=800E6;
{===>} frequency_2=800E6;
{===>} frequency_3=800E6;
{===>} frequency_4=800E6;
{===>} frequency_5=800E6;

{+ choice: true false +}
{===>} qerrset_1=true;
{+ choice: true false +}
{===>} qerrset_2=true;
{+ choice: true false +}
{===>} qerrset_3=true;
{+ choice: true false +}
{===>} qerrset_4=true;
{+ choice: true false +}
{===>} qerrset_5=true;

{===>} errmod_1="DIST";
{===>} errmod_2="DIST";
{===>} errmod_3="DIST";
{===>} errmod_4="DIST";
{===>} errmod_5="DIST";

{===>} err0_1=0.0;
{===>} err0_2=0.0;
{===>} err0_3=0.0;
{===>} err0_4=0.0;
{===>} err0_5=0.0;

{===>} err1_1=0.0;
{===>} err1_2=0.0;
{===>} err1_3=0.0;
{===>} err1_4=0.0;
{===>} err1_5=0.0;

{===>} err2_1=0.125;
{===>} err2_2=0.125;
{===>} err2_3=0.125;
{===>} err2_4=0.125;
{===>} err2_5=0.125;

{===>} err3_1=0.0;
{===>} err3_2=0.0;
{===>} err3_3=0.0;
{===>} err3_4=0.0;
{===>} err3_5=0.0;

{+ choice: true false +}
{===>} qexclude_1=true;
{+ choice: true false +}
{===>} qexclude_2=true;
{+ choice: true false +}
{===>} qexclude_3=true;
{+ choice: true false +}
{===>} qexclude_4=true;
{+ choice: true false +}
{===>} qexclude_5=true;

{+ choice: true false +}
{===>} qmove_1=true;
{+ choice: true false +}
{===>} qmove_2=true;
{+ choice: true false +}
{===>} qmove_3=true;
{+ choice: true false +}
{===>} qmove_4=true;
{+ choice: true false +}
```

```
{===>} qmove_5=true;

{===>} het1window_1=0.5;
{===>} het1window_2=0.5;
{===>} het1window_3=0.5;
{===>} het1window_4=0.5;
{===>} het1window_5=0.5;

{===>} pro1window_1=0.03;
{===>} pro1window_2=0.03;
{===>} pro1window_3=0.03;
{===>} pro1window_4=0.04;
{===>} pro1window_5=0.04;

{===>} het2window_1=0.5;
{===>} het2window_2=0.5;
{===>} het2window_3=0.5;
{===>} het2window_4=0.5;
{===>} het2window_5=0.5;

{===>} pro2window_1=0.05;
{===>} pro2window_2=0.05;
{===>} pro2window_3=0.05;
{===>} pro2window_4=0.04;
{===>} pro2window_5=0.04;

{================ relaxation matrix parameters ====================}
{* distance cutoff *}
{===>} relax_cutoff=5.0;
{* number of cutoff layers *}
{===>} relax_nlayers=3;
{* number of matrix doublings *}
{===>} relax_mdouble=10;

{================ restraint combination ====================}
{* Last iteration during which to use restraint combination as proposed by Peter
Guentert, for unambiguous long-range NOEs, set to -1 in order to switch it off *}
{+ choice: "-1" "0" "1" "2" "3" "4" "5" "6" "7" "8" +}
{===>} combination_last_it="-1";

{====================== iterations  ========================}
{* parameters for the 8 interations *}

{+ table: rows=8 "structures" "keep_structures" "assign_structures"
             "ambiguous_cutoff" "max_n" "violation_tolerance" "violation_ratio"
"window_weight"
   cols=9 "It0" "It1" "It2"  "It3" "It4" "It5" "It6" "It7" "It8" +}

{===>} structures_0=50;
{===>} structures_1=50;
{===>} structures_2=50;
{===>} structures_3=50;
{===>} structures_4=50;
{===>} structures_5=50;
{===>} structures_6=50;
{===>} structures_7=50;
{===>} structures_8=50;

{===>} keepstruct_0=0;
{===>} keepstruct_1=30;
{===>} keepstruct_2=30;
{===>} keepstruct_3=30;
{===>} keepstruct_4=30;
{===>} keepstruct_5=30;
{===>} keepstruct_6=30;
{===>} keepstruct_7=30;
{===>} keepstruct_8=30;

{===>} assignstruct_0=14;
{===>} assignstruct_1=14;
{===>} assignstruct_2=14;
```

```
{===>} assignstruct_3=14;
{===>} assignstruct_4=14;
{===>} assignstruct_5=14;
{===>} assignstruct_6=14;
{===>} assignstruct_7=14;
{===>} assignstruct_8=20;

{===>} ambigcutoff_0=1.01;
{===>} ambigcutoff_1=0.9999;
{===>} ambigcutoff_2=0.999;
{===>} ambigcutoff_3=0.99;
{===>} ambigcutoff_4=0.98;
{===>} ambigcutoff_5=0.96;
{===>} ambigcutoff_6=0.93;
{===>} ambigcutoff_7=0.90;
{===>} ambigcutoff_8=0.80;

{===>} maxn_0=20;
{===>} maxn_1=20;
{===>} maxn_2=20;
{===>} maxn_3=20;
{===>} maxn_4=20;
{===>} maxn_5=20;
{===>} maxn_6=20;
{===>} maxn_7=20;
{===>} maxn_8=20;

{===>} violtoler_0=1000.0;
{===>} violtoler_1=1000.0;
{===>} violtoler_2=1.0;
{===>} violtoler_3=0.5;
{===>} violtoler_4=0.1;
{===>} violtoler_5=1.0;
{===>} violtoler_6=0.1;
{===>} violtoler_7=0.1;
{===>} violtoler_8=0.1;

{===>} violratio_0=0.5;
{===>} violratio_1=0.5;
{===>} violratio_2=0.5;
{===>} violratio_3=0.5;
{===>} violratio_4=0.5;
{===>} violratio_5=0.5;
{===>} violratio_6=0.5;
{===>} violratio_7=0.5;
{===>} violratio_8=0.5;

{===>} window_weight_0=1.0;
{===>} window_weight_1=1.0;
{===>} window_weight_2=1.0;
{===>} window_weight_3=1.0;
{===>} window_weight_4=1.0;
{===>} window_weight_5=1.0;
{===>} window_weight_6=1.0;
{===>} window_weight_7=1.0;
{===>} window_weight_8=1.0;

{================ parallel jobs ====================}
{* How many nodes do you want to use in parallel? *}
{* leave unused fields blank, make sure that the queues are actually running *}
{+ table: rows=10 "1" "2" "3" "4" "5" "6" "7" "8" "9" "10"
 cols=3 "queue command" "cns executable" "number of jobs" +}

{===>} queue_1="csh";
{===>} cns_exe_1="/usr/site/cns_solve_aria/intel-i686-linux/bin/cns";
{===>} cpunumber_1=2;

{===>} queue_2="";
{===>} cns_exe_2="";
{===>} cpunumber_2=0;
```

```
{===>} queue_3="";
{===>} cns_exe_3="";
{===>} cpunumber_3=0;

{===>} queue_4="";
{===>} cns_exe_4="";
{===>} cpunumber_4=0;

{===>} queue_5="";
{===>} cns_exe_5="";
{===>} cpunumber_5=0;

{===>} queue_6="";
{===>} cns_exe_6="";
{===>} cpunumber_6=0;

{===>} queue_7="";
{===>} cns_exe_7="";
{===>} cpunumber_7=0;

{===>} queue_8="";
{===>} cns_exe_8="";
{===>} cpunumber_8=0;

{===>} queue_9="";
{===>} cns_exe_9="";
{===>} cpunumber_9=0;

{===>} queue_10="";
{===>} cns_exe_10="";
{===>} cpunumber_10=0;


{====================== SA protocol ========================}
{* type of molecular dynamics *}
{+ choice: "torsion" "Cartesian" +}
{===>} md_type="Cartesian";

{* initial seed for random number generator *}
{* change to get different initial velocities *}
{===>} iniseed=89764443;

{* initial temperature for TAD *}
{===>} tadinit_t=10000;
{* initial temperature for TAD and Cartesian dynamics *}
{===>} carinit_t=2000;

{* final temperature after first cooling step *}
{===>} final1_t=1000;

{* finale temperature after second cooling step *}
{===>} final2_t=50;

{* Cartesian time step *}
{===>} timestep=0.003;
{* factor for timestep and number of steps in TAD *}
{===>} tadfactor=9;

{* initial number of MD steps *}
{===>} initiosteps=10000;

{* number of MD steps for refinement *}
{===>} refinesteps=4000;

{* number of MD steps during first cooling stage *}
{===>} cool1_steps=5000;

{* number of MD steps during second cooling stage *}
{===>} cool2_steps=4000;

{* first iteration for Cartesian refinement (after TAD) *}
```

```
{===>} cart_firstit=0;

{* Sort structures accordingly to total energy or sum of restraints energies? *}
{+ choice: "totener" "restener" +}
{===>} filesort="totener";

{====================== final water refinement  ========================}
{* Do you want water refinement for the last iteration? *}
{+ choice: "yes" "no" +}
{===>} firstwater="yes";

{* Which solvent do you want to use? *}
{+ choice: "water" "dmso" +}
{===>} solvent="water";

{* number of structures for the water refinement *}
{* refine the n best structures regarding energy  *}
{===>} waterrefine=20;

{====================== procheck, whatcheck and prosa analysis of the last iteration
======}
{* Make sure that your PRODIR and PROSA_BASE system variables are set correctly. Leave
fields empty if you don't want to perform these checks   1.  procheck executable *}
{===>} procheck_exe="";
{* 2. procheck_comp.scr script *}
{===>} procheckcomp_exe="";
{* 3. whatif executable *}
{===>} whatif_exe="";
{* 4. prosaII executable *}
{===>} prosa_exe="";
{* number of pdb files for analysis *}
{===>} how_many_pdb="20";

{=========================================================================}
{        things below this line do not normally need to be changed        }
{=========================================================================}

set message on echo on end
```